P32 movement through a freshwater laboratory ecosystem

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P^{32} MOVEMENT THROUGH A FRESHWATER LABORATORY ECOSYSTEM

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INTRODUCTION

A comparatively new field of ecological research involving radioisotopes has been developed in recent years as a result of the pioneering efforts of a number of ecologists (Hutchinson et al (1948) (1950), Coffin et al (1949), Hayes et al (1951)(1952), Macarter et al (1952), Rigler (1956)(1961), Kuenzler et al (1958), Odum, E.P. (1961), Odum, E.P. et al (1963)(1963), and many others). Radioecology is concerned with the study of radioactive substances and radiation in a number of environmental contexts. There are two distinct phases or branches of radioecology: on the one hand concerning the effects of radiation on individual populations, communities and ecosystems, and on the other hand concerning the fate of radioactive substances released into the environment and the manner in which natural communities affect the distribution of these materials. (This study is concerned with the latter category.)

The continued accelerating use of nuclear power for peaceful purposes over the last decade or so, as fossil fuel supplies become depleted or obsolescent, has produced the necessity for more detailed research on the effects and behaviour of radioactive wastes dispersed into the various realms of the biosphere, especially aquatic ecosystems (Higgins (1949), Foster and Davis (1955), Davis and Foster (1958), Templeton (1959), Rice (1963), Alexander (1965), Comar (1965), Reichle et al (1965), Reichle (1967), Pomeroy and Kuenzler (1969)). When radioactive nuclides (isotopes) are released into the environment they invariably become diluted and dispersed through the system by natural processes. They may also be concentrated into living organisms during food chain transfers (i.e. biological magnification) or localised into particular ecosystem "components", e.g. muds, sediments, organic detritus or soils. Depending on their half lives these isotopes may become harmless within a short period of time or,

in the case of longer lived isotopes, e.g. ^{90}Sr etc., pose a long term threat to the viability and stability of whole ecosystems or important parts thereof. (Townsley et al (1960)(1961), Ophel (1962), Templeton (1962)(1965), Menkley et al (1963), Sabo et al (1963), Osterberg et al (1964), Paakkola (1965))

Food chains are particularly important since they tend to select for isotopes of the essential elements, e.g. ^{32}P, ^{45}Ca, ^{131}I, and to select against non-essential elements. Thus the biological hazard presented by radioisotope contamination depends primarily on their availability to living organisms and the half life of the isotope involved. Some of the earliest data on the concentrative effects of both aquatic and terrestrial food chains were obtained by radioecologists at a variety of nuclear plants contaminating local land-water environments in the United States, e.g. AEC Hanford plant on the Columbia River, Washington, (Herde (1947)(1947), Coopey (1948), Herde et al (1950), Berry et al (1950), Coopey (1951), Davis and Cooper (1951), Olson and Foster (1952), Foster and Rostenbach (1954), Davis et al (1956), Hanson and Kornberg (1956), Davis and Foster (1958), Ophel (1962))and the Oak Ridge National Laboratory, Tennessee (Whiteoak Lake and Clinch River), (Knobf (1951), Helm (1953), Krumholz (1956)(1961), Krumholz and Foster (1957), Krumholz et al (1957), Nelson (1967)).

More basic research into transfer routes, concentration effects and on the basic functioning of ecosystems which underlie radioecological phenomena will be of distinct importance as Man enters the age of the "fast breeder" reactor of the late twentieth century.

^{32}P is a radioisotope of metabolic and ecological importance which has proved to be a useful tool in a wide variety of research fields, e.g. trophic relationships, mineral cycling, community metabolism, energy flow and modes of uptake and loss (^{32}P or phosphorous

compounds) by different organisms. The importance of ^{32}P and other radiotracers in ecological studies has been reviewed by a number of authors (Jenkins and Hassett (1950), Comar (1955), Dahms (1957), Klement and Schultz (1962, 1963, 1964, 1965), Anon (1963), Deevey et al (1963), Odum, E.P. and Golley (1963), Schultz and Klement (1963), Hungate (1966), Polikarpov (1966), Nelson and Evans (1969)).

Tracers in general are extremely useful when tracing biogeochemical cycles and in inferring flux rates in steady state systems. The elements of nature vital for life are almost never homogenously distributed, nor present in the same chemical form throughout an ecosystem. Rather, materials exist in pools or compartments with varying rates of exchange between them, and it is in this context that tracer experiments have produced major advances in our knowledge of biogeochemical cycles. Tracers may offer useful means for solving two major problems in ecosystem functioning, i.e. relating the one way flow of energy to the cycling of materials, and of determining how physical and biological factors interact to control the ecosystem (Odum, E.P. (1961), Odum, E.P. and Golley (1963)). They are also becoming important tools in the study of community metabolism, e.g. ^{14}C use in production measurements in aquatic ecosystems. (Steeman-Nielsen (1952), Allen (1963), Thomas (1964))

Odum, E.P. and Kuenzler (1963) have pointed out their usefulness in determining trophic positions and mapping food webs. (Pendleton and Grundmann (1954), Pendleton and Smart (1954), Ball and Hooper (1963), Crossley (1964), Reichle and Crossley (1965), De La Cruz and Wiegert (1967), Wiegert and Odum, E.P. (1969)) Shure (1970) has however recently pointed out some of the limitations involved when using radiotracers to determine consumer trophic positions.

Odum, E.P. (1961) has also suggested the possible use of radioisotopes as "activity meters" in the field. He points out that a

major bottleneck in energy flow studies arises from the inability of ecologists to measure the activity and metabolic rates of organisms in the field as the functioning parts of intact ecosystems. This applies particularly to heterotherms which possess no real basal metabolic rate (i.e. it varies within fairly wide limits according to a number of factors). Therefore laboratory data can only be applied very cautiously to field data. Odum, E.P. (1961) has postulated that by measuring the loss rates of certain suitable isotopes from species organism in the field a measure of the metabolic rate can be gained, if the loss rate can be linked to metabolic rate or some specific aspect of metabolic activity.

Phosphorous is an essential element in the chemical makeup of protoplasm from which all living organisms are formed. Its fundamental importance to living organisms, linked with its relative scarcity in most environments (especially aquatic ones) makes it probably the most important factor limiting productivity in many regions of the biosphere.

The importance of phosphorous to living organisms and ecosystem dynamics in general makes it one of the elements of most concern where radiochemical contamination from atomic installations is concerned. Fortunately, both from the view of research purposes and radiocontaminative processes, it has a relatively short half life of 14.5 days and emits only moderately high energy (1-3 Mev) β particles; although if released in large enough quantities it could be viewed as a radioecological hazard.

The usefulness of radioisotopes in scientific research arises from the fact that the isotope atoms behave chemically and biologically in essentially the same way as the atoms of the non-radioactive isotopes of the element, with the advantage that these "tagged" atoms, their movements and rates of movement, modes of transport

and locii of concentration can be measured quantitatively. This information on isotope movement can be used to determine quantitatively the patterns of movements of the non-radioactive stable forms, provided the isotopic dilution of the non-radioactive to radioactive isotope atoms in the various system components are known (i.e. the extent to which the ^{32}P atoms in any system or fraction are diluted with stable ^{31}P atoms, that is the fraction of ^{32}P atoms among all phosphate atoms).

^{32}P isotope tracer studies in the last twenty-five years have elucidated much about the routes, and more importantly, the rates of movements back and forth between the various "phases" of aquatic ecosystems, such as small freshwater ponds and lakes. Since these are relatively self-contained (i.e. closed systems) they provide good sites in which to study inorganic nutrient cycling.

The use of ^{32}P in the investigation of aquatic ecosystems was pioneered by Hutchinson and Bowen (1948), Coffin et al (1949), Hutchinson (1950), Hayes and Coffin (1951), Hayes et al (1952), Hayes and Jodrey (1952) and Rigler (1956). Pomeroy (1970) has reviewed the various studies carried out on ^{32}P cycling in lakes: the main conclusions of these studies can be briefly reviewed as they have some bearing on this paper.

1. It was Hutchinson (1941) who recognised that the amount of phosphate present at certain times in lake waters was insufficient to account for many of the biological processes taking place. This finding was later verified by the ^{32}P tracer studies of Hutchinson and Bowen (1948), Hutchinson (1950), Hayes (1951).

It has been found in most studies that phosphates do not move evenly and smoothly from organism to the environment and back again. At any one time most of the phosphate in aquatic ecosystems is tied up in living biomass or solids, e.g detritus and the organic and

inorganic particles of the sediment. No more than 10% (Chu (1949) has pointed out that not all of this may be in a chemical form which is directly accessible to the organism, especially plankton and macrophytes) is likely to be in a soluble form at any one time. Hayes (1951) has pointed out that a pond or lake is not merely a body of water containing nutrients, but an equilibrated system of water and solids in which, under ordinary conditions, nearly all of these nutrients are in a solid state or stage. It is the rate of release of these nutrients, e.g. phosphorous, nitrogen, carbon, from the solids, along with the solar input, seasonal temperature cycle and various climatic factors, which are the important processes regulating the rate of function of the ecosystem on a day to day basis.

2. A rapid back and forth movement or exchange occurs all the time, but extensive movement between the solid and dissolved states is often jerky or irregular with periods of nett release from sediments etc., followed by periods of nett uptake by organisms or sediments depending largely on temperature conditions and organism activities. Generally speaking, uptake rates are more rapid than release rates; plants, for example, will take up phosphorous compounds in the dark or under other conditions when they cannot immediately use it. During periods of rapid growth, e.g. spring, all available phosphates may become bound into producers and consumers. The system may then have to "slow down" until bodies, feces, etc. can be decomposed and these phosphates again released and recirculated. Thus the concentration of phosphate etc. in water at any one time may bear little relationship to production, e.g. a low level of phosphate could indicate an impoverished or highly active system (metabolically speaking): only by measuring flux rates can the true situation be determined. The rapid flux of phosphate is a typical characteristic of highly

productive systems and it is this flux which is more important than the gross concentrations present in maintaining high rates of organic production.

3. The turnover times of dissolved inorganic phosphate were found to be shorter than was first envisaged by limnologists, i.e. minutes instead of days, e.g. 3.6-5.4 minutes for water to plankton (Rigler (1956)(1961)). Rigler (1956) and others (Hayes (1953), Harris (1957), Whipple (1901)) have pointed out that bacteria are largely responsible for this rapid flux in the plankton. Rigler found that approximately 85% of introduced ^{32}P was removed from solution into suspended lake bacteria within thirty-two hours, and concluded that they play a major part in lake PO_4 circulation. Pomeroy (1963) has found, on the other hand, that bacteria have a significant but very variable influence in marine water phosphate cycling.

4. Whittaker (1954) and Rigler (1956) have noted that phosphorous (inorganic) uptake tends to be most rapid in phosphorous impoverished lakes.

Many outdoor ecosystems are complex, hard to delineate, often difficult to study and, in the case of radiotracers, (in this country at least) unavailable for investigation. Thus traditional scientific methods of experiment and control are difficult to enforce. A recent approach to these problems involves the use of simplified field systems in the laboratory with discrete boundaries which can be replicated at will and used to test ecological principles (Whittaker (1953, 1954, 1961, 1971), Foster (1959), Byers (1962, 1963, 1964, 1965)). There are two basic types:

a) i) Axenic cultures (Odum, H.T. and Hoskins (1957), Byers (1963)). These are produced by multiple seeding of organisms from natural habitats into laboratory situations.

ii) Polyaxenic cultures: more sophisticated versions of i) (Nixon (1969), Taub (1969)(1969)).

b) Gnotobiotic systems (Daugherty (1959): here the exact composition down to bacteria is known.

Whittaker (1954)(1961) used axenic cultures developed in the laboratory as models on which to study problems of radioisotope contamination in natural aquatic environments. Byers (1963) has used similar techniques to investigate the metabolism of simple aquarium microcosms.

Lastly, it is perhaps worth mentioning another technique which, in conjunction with tracer techniques, can be applied to the investigation of nutrient cycling. Relatively small amounts of field data can be used to construct simplified but realistic models of element cycling through natural systems. This is achieved by using a system of differential equations (Whittaker (1961), Odum, H.T. (1960)) to describe the manner in which the isotope (i.e. element) contents and movements through one compartment or phase of an ecosystem affect those of adjacent parts of the system.

Such models can be manipulated by computers (digital and analog) to determine the transfer coefficients which will make the model a more realistic mimic of the real situation. This technique has been effectively used in conjunction with field and laboratory data on ^{32}P and ^{14}C movements (Odum, H.T. (1960), Whittaker (1961), Garfinkel (1962), Neel and Olson (1962), Olson (1963), Pomeroy, Johannes, Odum, E.P. and Roffman (1966)(1969)) to produce quantitative pictures of element cycling various ecosystems and the effect of changing physico-chemical conditions on these cycling patterns.

AIMS

Laboratory ecosystems can be useful in demonstrating the great value of radioactive isotopes in the study of various environmental phenomena. Tracers "extend" Man's powers of observation with regard to biological function within the community and the framework of individual species by enabling him to "observe" otherwise undetectable processes and events. This study was carried out for the following reasons.

a) ^{32}P was a reasonably safe and easy isotope to work on with the facilities to hand. It is a major macronutrient of great importance to living organisms and is invariably found in short supply, especially in aquatic communities.

b) This study was aimed at elucidating and illustrating the major movements of phosphorous through a freshwater pond community set up in an aquarium microcosm.

c) ^{32}P can assume an important part of the aquatic contamination produced by certain reactor plants and their effluents (e.g. Hanford works and Oak Ridge National Laboratory, U.S.A.), and it was hoped that this study would illustrate some of the possible routes of ^{32}P contamination and its ultimate fate within such an environmental framework.

d) Other facets of the study involved the use of radiotracer information to produce estimates of production efficiency and the investigation of uptake/loss processes in two species of aquatic invertebrates.

METHODS.

PART ONE.

THE AQUARIUM MICROCOSM.

1. INTRODUCTION.

The experiment was carried out in a glass sided, steel framed aquarium 182 cm. long, 24 cm. wide and 32 cm. deep. On 11th May 1972 the aquarium was filled to a depth of 28 cm. with water from a nearby set of ponds at Willington, Co. Durham, (O.S.S. 85 204 343). On the same day the bottom of the aquarium (surface area 4,368 cm^2) was covered to a depth of approximately 5 cm. with 12 Kg (dry weight) of organic mud removed from the above ponds. The mud was thoroughly stirred in an attempt to spread the lighter organic material as evenly as possible over the heavier inorganic substrate. The mud layer was later sampled with the aid of plastic slide boxes (twelve with a volume of 19 $cm.^3$ and thirty-six with a volume of 15 cm^3), which were placed in four larger (14 cm. x 11 cm. x 3 cm.) plastic trays and covered with mud. On the next day, forty-six glass slides (sixteen with a surface area of 75 cm^2 and thirty with a surface area of 60 cm^2) were suspended vertically in the water at various depths by nylon lines stuck to the outside of the aquarium. These slides were later used to samole the sidewall community growing on the exposed glass walls of the tank, above the mud. The total glass area available to this community was 14,927 cm^2, of which the sample slides made up 2,978 cm^2. The volume of water introduced to the tank was calculated to be 122.304 litres; this effectively remained constant through the period of the experiment, since glass covers prevented evaporative loss. The tank was constantly aerated by a 3 cm. air cube suspended about 5 cm. below the water level at one end of the tank. The air pressure was adjusted to produce a gentle circulation in the upper layers of the tank. At no time was the aeration

pressure sufficient to cause any noticeable sediment circulation from the mud into the upper water layers.

The tank received an approximately equivalent light exposure on all sides; an overhead fluorescent lamp was used to compensate for the aquarium's shady position in the laboratory. During the course of the experiment the water temperature varied between 17°C at night and a maximum of 21.5°C in the day. The simple system set up was, along with other fractions added at later dates, allowed to develop for three weeks prior to the addition of a P^{32} "spike".

During the three weeks prior to the addition of the "spike", a representative cross section of plant and animal life was removed from the aforementioned ponds and gradually transferred to the aquarium microcosm.

The first addition was Callitriche sp., a large mass of which was transplanted into the aquarium (along with its roots and attached mud). The majority of animal species used were transferred to the aquarium during the week preceding the addition of the P^{32} spike. A number of smaller organisms, e.g. Polycelis nigra (Planarian), Planorbis alba and Limnaea pereger (Mollusca), Leptocerus sp. and Triaenodes sp. (Caddis larvae), Hydrobius sp. (Coleoptera), Hydracarina (Water Mites); Limnesia fulgida, Diplodontus despiciens, Hygrobates longipalpis and Hydrarachna globosa, were gradually transferred into the aquarium up to three weeks before P^{32} addition. Larger herbivores, e.g. Rana sp. tadpoles, Limnophilus stigma and Limnophilus xanthodes (Caddis larvae), Corixa punctata (Hemiptera) were added about four days before "spiking". Daphnia pulex were added twenty-four hours before "spiking" along with the filamentous alga, Spirogyra sp.

The carnivores, e.g. Dytiscus sp. larvae and adults, Hyphydrus ovatus and Agabus sp. adults (Coleoptera), Sialis sp.larvae (Alder fly), Enallagma sp. (Damsel fly nymphs), Gerris lacustris (Pond Skater), Erpobdella octoculata (Leech), Nepa cinerea and Notonecta glauca (Hemiptera) and Gasterosteus aculeatus (sub species gymnurus) (Stickleback) adults and fry were added last of all.

All the animals introduced into the aquarium were selected to be of approximately the same size (within each species) in order to try and reduce to a minimum the individual biological variation in P^{32} uptake during the experiment. Apart from Daphnia sp., the total number of each species introduced was known. During the period between removal from the pond and transfer to the aquarium the animals were kept in two hundred litre plastic containers along with mud, aquatic plants and, in the case of carnivores, their usual food species. Practically every species of larger animal found in the pond was represented in the aquarium, and later sampled.

The final water level was recorded at the time of "spiking" and was not observed to decrease appreciably during the course of the experiment. Immediately prior to the addition of the P^{32} "spike", a glass tube (surface area 10.2 cm^2) was placed in the centre of the tank in order to estimate the rate of P^{32} sedimentation processes occurring during the experiment.

1.1. Sampling Procedures.

The experiment commenced at 11 a.m. on 2nd June 1972 when 39.06 uc (86.72×10^6 c.p.m.) of P^{32} was introduced and mixed into the surface layers of the aquarium water. Beginning one hour and ending 1200 hours (fifty days) later, samples were removed from all the major community

fractions (water, mud, sediments, sidewalls, plants and animals). Samples were removed at 1, 7.5, 22, 53, 168, 363, 555, 864 and 1200 hours after the P^{32} was introduced. In a number of cases further samples were removed at 72, 96, 103, 119 and 196 hours after P^{32} introduction. The sampling of some organisms was discontinued before 1200 hours owing either to shortage of material or difficulty in sampling.

Since what occurs after the introduction of P^{32} happens rapidly at first and then progressively more slowly as time passes, a large proportion of the sampling points were concentrated into the first 168 hours of the experiment.

The following samples were removed at most sampling points:

1.1.1. Sixty ml. of aquarium water, ten ml. of which was immediately transferred to the counting apparatus and its radioactivity (in c.p.m.) recorded. The remaining fifty ml. was vacuum filtered through a 25 mm.diameter millipore filter paper (pore size 0.45 u). Ten ml. of the resulting fluid was then transferred to the counting apparatus and its radioactivity recorded. Towards the end of the experiment the radioactivity of the filtered fraction declined to very low levels; as a result larger volumes of filtered water were used (fifty or hundred ml.) in counting. These were evaporated down to ten ml. volumes and then counted on the recording apparatus. The millipore filters were

carefully removed, transferred to watch glasses and placed in a drying oven.

1.1.2. A number of glass plates were removed from different points in the aquarium (the sidewall area sampled varied from 120 to 195 cm^2 on most occasions), quickly rinsed in distilled water to remove any aquarium water and then transferred to the drying oven. Any higher organisms, e.g. snails, eggs and insect larvae, attached to the slides, were removed before drying.

1.1.3. Plant and animal samples were removed from the tank, killed in hot water and washed under a cold water jet for about two minutes in order to remove any P^{32} adhering to their surfaces. The snails, Limnaea pereger, were removed from their shells and the two parts of the organism were treated separately. The caddis fly larvae, Limnophilus stigma, Limnophilus xanthodes, Leptocerus sp. and Triaenodes sp. were removed from their cases, which were then discarded. Samples were then transferred to the drying ovens in watch glasses. Animal samples were removed with a small net; plant samples were picked out and cut off at a variety of points with scissors and forceps.

1.1.4. Sediment samples were removed after longer intervals (0-7, 7-36, 36-50 days) in the tank. The sample tube was allowed to stand for an hour, after which the water above the fine sediment was

removed with a pipette. The sediment was rinsed out of the tube, evaporated and dried in the oven.

1.1.5. Mud samples were removed from the aquarium floor with a hand operated grab. The volume of mud removed varied from 30 to 57 cm^3 on most occasions. Living organisms were returned to the tank if found in the mud sampler cases. The mud was tipped into large watch glasses and the insides of the sample box washed off and added. The mud was then transferred to the oven for drying.

All samples were dried to constant weight in a drying oven set at 70°C. Most animal and plant samples required at least seventy-two to ninety-six hours drying, some, e.g. mud, required at least a week.

After drying, all samples were weighed on a balance accurate to four decimal places. Samples were allowed to equilibrate with the laboratory humidity and temperature for about one hour after removal from the oven. It was noticed early in the weighing procedure that immediate weighing after removal from the oven gave erratic results. This was due to the rapid condensation of water vapour on the sample and watch glass surfaces as cooling occurred. In the smaller samples this would have produced an appreciable error, if it had not been noticed and allowed for, as most of the animal sample weights were well below one gram.

1.2. Biomass Estimates.

1.2.1. Plants.

Prior to transplanting, the Callitriche and Spirogyra were weighed wet. A number of known wet weight samples were removed, dried for four days at 70°C and reweighed. Since the total wet weight was known and the wet to dry weight conversion factor calculated, the total dry weight introduced can be estimated fairly accurately. The wet to dry weight ratios were respectively: Spirogyra sp. 18:1, Callitriche sp. 12:1.

1.2.2. Animals.

The precise numbers of each species introduced was known; the average dry weight per individual of a species was calculated on the basis of the total sample weights and the numbers removed by the end of the experiment. In a number of cases the precise weight of a species fraction was known, e.g. Dytiscus sp. adults and larvae, Notonecta, Nepa, Erpobdella, Sialis and Gasterosteus adults, because by the end of the experiment all the introduced specimens had been recovered. The Daphnia biomass was estimated on the basis of the introduction of a known wet weight and the later determination of the wet to dry weight ratios.

1.2.3. Sidewall Community.

At each sample time the dry weight per unit area of the sidewall community on the glass sample

plates was determined by weighing the dried plates before and after the acid digestion process. Since the total glass area available to this community fraction was known, the total tank biomass could be estimated at different points in time through the course of the experiment. (Fig.9, Table 6).

1.2.4. Millipore Fraction.

During the experiment fifty ml. water samples were filtered through millipore filters at various times in order to determine the P^{32} uptake by this fraction. The filter papers used were dried to a constant weight before filtering. Since the dry weight of the filter paper plus the millipore fraction filtered out from a known volume of water can be measured, the biomass per ml. can be determined, and from this the biomass held in the 122.3 litres of water in the aquarium. (Fig.9, Table 5). The very small sample weights involved make these estimates rough ones at best.

1.2.5. Mud Fraction.

The mud used was weighed wet prior to its introduction into the tank. Nearly two hundred grams wet weight was removed and dried and the wet to dry weight ratio calculated as before; from this the total dry weight introduced can be estimated.

1.2.6. Sediment Accumulation.

The sediment sampler was removed at intervals and the dry weight of organic matter sedimenting into the sampler during the preceding period was measured. The total amount sedimenting into the mud was estimated by multiplying the sample weight by $\frac{4,368\ cm^2}{10.183\ cm^2}$, i.e. 428.95. The 4,368 cm^2 refers to the mud surface area, and the lower figure to the surface area of the sample tube open to the water.

Finally, biomass estimates were made at the end of the experiment by removing all the remaining organisms and determining their dry weights.

1.3. Digestion Techniques.

A number of methods can be used in the digestion of samples and tissues in preparation for biochemical and radiological analysis, (McCarter and Steljes (1948), Krumholz (1953) and Robinson (1941)). The digestion technique used (along with a number of minor alterations) was one originally developed by Krumholz (1953) for the routine preparation of fish tissues, in order to determine their gross beta radioactivity.

The method was as follows:

1.3.1. The dried, weighed, samples (animals, plants, and millipore filter) were placed in 25 ml. test tubes calibrated at 8 ml. volume.

1.3.2. Concentrated nitric acid was added (5-6 ml.) and the samples allowed to stand for twelve to twenty-four hours.

1.3.3. When the bulk of the organic tissue had been dissolved, the samples were heated gently to between 40-50°C for two to four days until organic tissue had been reduced to a minimum.

1.3.4. After two to four days digestion, the samples were allowed to cool and one to four drops of a thirty per cent hydrogen peroxide solution were added to oxidise any remaining organic material. Caution was required at this point as excess hydrogen peroxide caused the samples to boil over.

1.3.5. Heating was resumed after about an hour and hydrogen peroxide was added, a few drops at a time, until the solution became clear and free of organic material. The attainment of a clear solution indicated that the tissue digestion was complete and that all the organic material had been oxidized.

1.3.6. The samples were allowed to cool, made up to the 8 ml. mark with nitric acid and then transferred to the liquid counter with a pipette. The remaining 2 ml. required to fill the liquid counter to the standard mark, were made up with nitric acid used to carefully wash out any P^{32} adhering to the sides of the sample tube. The liquid counter was rotated gently through 360° a number of times in order to thoroughly mix the sample liquid prior to counting.

A number of difficulties were encountered when using this technique which may have had some effect on the accuracy of the sample counts.

1.3.7. (a). Plant tissue was generally very difficult to digest completely and usually required in excess of 20 ml. of concentrated nitric acid. At the end of digestion there was always a small amount of tissue remaining; however, by this time all the P^{32} fixed in the tissue was assumed to have been extracted and dissolved in the acid.

(b) Some of the animal species also resisted complete digestion, namely Limnaea pereger, Rana tadpoles, Limnophilus stigma and Limnophilus xanthodes. In the latter two species the undigested portion consisted largely of fats which hold little P^{32} after digestion (Krumholz, 1953). The former two species produced appreciable amounts of mucus, fats and pigmented tissue which generally formed a rind around the edge of the tubes. This was considerably reduced by adding concentrated sulphuric acid in equal volumes to the nitric acid.

The digestion of sidewall plates, mud and sediment samples required a different technique. Sidewall plate communities were rapidly digested overnight in a mixture consisting of equal parts of concentrated nitric and sulphuric acids. Any P^{32} adhering to the plates was washed off with

concentrated nitric acid when digestion was completed. The volume of acid required for complete digestion usually exceeded 100 ml; a 10 ml. aliquot of this was taken and used to determine the total sample count.

The dried mud samples were invariably divided into a number of portions which were digested separately. The original sample was ground up into a fine state of division with a mortar and pestle. Digestion was carried out with mixtures of concentrated nitric acid and sulphuric acid. The acid volumes required for digestion varied from 30 to over 100 ml; digestion proceeded for up to fourteen days with regular mixing. The clear liquid remaining was poured off and centrifuged to sediment out any fine mud and other particles . 10 ml. portions of the fluid samples were assayed in the liquid counter in the usual manner. This washing procedure was repeated two or three times until the 10 ml.aliquots assayed on the counter recorded a negligible number of P^{32} disintegrations. This was taken to indicate that all the P^{32} in the mud sample had been removed and measured. It is interesting to note that, as successive washings were assayed, the P^{32} activity of each washing did not always decrease as one might expect.

It was originally assumed that a major proportion of the P^{32} in a mud sample would be removed in the first acid washing. This rarely occurred, and suggests that an appreciable fraction of the samples P^{32} was held in a tightly bound form with other mineral compounds in the mud.

Sediment samples were digested in a similar way to the plant and animal specimens; only here larger volumes of acid were employed.

1.4. Counting Techniques.

The majority of samples (80% +) were assayed using a Mullard Veal liquid counter (volume 10 ml.) mounted in a one inch thick lead castle connected to a Scaler 1800E recording unit. The remaining twenty per cent of samples were assayed in the same liquid counter connected to a Ratemeter, or in a 6 ml.unshielded liquid counter connected to a Decade type recorder.

The radioactivity of the acid digest samples was then determined in the following manner:

1.4.1. The background radiation level (i.e. counts per minute) was determined by using a liquid blank in the counting apparatus. This blank was a one per cent sodium hypophosphate solution.

1.4.2. The radioactivity of the acid digest sample was then determined.

1.4.3. After each acid digest had been assayed the liquid counter was thoroughly washed out several times with concentrated nitric acid, then a hypophosphate solution and finally water.

Thorough washing was vital in order to reduce P^{32} contamination of the counter to a minimum. Contaminated counters will result in a high background count and this will reduce the efficiency and accuracy with which samples with a low level of radioactivity can be measured.

A number of correction procedures must be made before the total radioactivity of a sample can be determined.

1.4.4. The background count was subtracted from the gross sample count (i.e. sample count plus the background count) to give the actual counts per minute produced by the sample alone. This was required because the sample count recorded consisted of disintegrations produced by the sample and disintegrations produced by background activity from a variety of sources, e.g. cosmic rays, nearby radioactive materials, natural radioactivity produced by the materials from which the apparatus was made and radioactive contamination of the liquid counter by previous samples.

1.4.5. The resulting figure was corrected for radioactive decay since the time of P^{32} introduction to the tank, i.e. zero hours, as it was the movements and relative concentrations of P^{32} which were the concern of this experiment and not the P^{32} levels as affected by decay. This correction was required because P^{32} has a fairly short half

life of 14.3 days (363 hours) i.e. after two weeks fifty-one per cent of the original P^{32} introduced into the system remains, after four weeks twenty-six per cent, after six weeks thirteen per cent, and after eight weeks 6.6%.

1.4.6. This figure (counts per minute at zero hours) was then converted to counts per minute per gram of the samples dry weight.

1.4.7. In a number of cases the digest volume exceeded 10 ml. In these cases the total acid digest volume was measured, a 10 ml. aliquot was taken and assayed, the background count was subtracted from this and the result was multiplied by the original sample volume divided by ten. For example, a 10 ml. aliquot taken from a sample with a volume of 18 ml. recorded a nett count of 100 c.p.m., therefore the total sample count is given by $\frac{18}{10} \times 100 = 180$ c.p.m.

1.4.8. The final calculation made is one which corrects for the effects of the counter efficiency. Due to the design of the liquid counter and the materials from which it is constructed, a large (but relatively constant) proportion of the disintegrations produced by the samples are not recorded by the electrodes of the liquid counter. It is possible to correct for this phenomenon by assaying a 10 ml. sample with a known activity. The counter efficiency is then given by:

$$\frac{\text{Nett recorded c.p.m. in sample}}{\text{Actual c.p.m. produced}} \times 100$$

The counting efficiency of the Scaler system was almost exactly ten per cent. The other systems used gave lower counting efficiencies and these were determined at intervals of a few days, thus any fluctuations in efficiency could be determined and allowed for when determining the actual activity density of a sample.

1.5. Statistical Considerations.

These were concerned with expressing the reliability or accuracy of the observed count rate in terms of the accuracy with which the sample was measured by the recording apparatus. The accuracy which can be achieved in any measurement is dependent on the magnitude of the count rate and the time taken over the observation. This is due to the random nature of the events recorded, i.e. several determinations of the count rate made under identical physical conditions will produce a number of values scattered about a mean. The Poisson distribution shows that the larger the number of individual determinations made, the nearer the mean value will be to the "true value".

In all cases the Standard Error of the sample counts were determined. Standard Errors were calculated in two ways.

1.5.1. Where a single determination was made the Standard Error was calculated as:

$$S.E. = \sqrt{\text{Total number of counts recorded}}.$$

1.5.2. In those cases where a single determination of the counts recorded over a set time was made a

number of times, the Standard Error was given as:

$$S.E.^2 = \sum \frac{(n-\bar{n})^2}{x(x-1)}$$

$\bar{n}$ = mean value.

n = sample count.

x = number of sample counts taken.

These two methods were used because it is more accurate to make a large number of individual determinations of the sample count rate where it is only marginally higher than the background count rate. This gives slightly greater accuracy since it puts greater weighting on the theoretically higher proportion of individual values which approximate to the mean value, and enables one to reject any determinations which appear to be more inaccurate than is expected purely on probability theory. In those cases where the sample count rate exceeded the background rate by a factor of ten times, it is more convenient and no less accurate to set the counting machine a large number of pulses, e.g. 10^4 to 10^6 to record in one run only.

During the early stages of the experiment in particular, many of the samples were producing low count rates and therefore required a large number of individual determinations for accurate assaying. In most cases the Scaler was set to record a set number of counts (usually four hundred or one thousand) and this was repeated

five to twenty times for each sample.

In biological work the individual biological variation between different individuals or organisms is sufficiently large to make the attainment of a Standard Proportional Error of two per cent, all that is required in radioactivity determinations. When determining the count rate of a sample the Standard Proportional Error can be reduced to any desired dimension in a single determination by continuing the count for a long enough period of time. For example, if 10^4 counts are recorded for a sample, no matter what time period is required, the Standard Error is: $\sqrt{10^4}$

$= 100$

that is one per cent.

In most cases in this experiment, a Standard Proportional Error of at least two to three per cent was obtained, i.e. a minimum of 2,500 counts was recorded per sample. In a few cases, especially those samples with a low count rate, the Standard Proportional Error was larger than three per cent because insufficient counts were recorded.

In the majority of cases a total of at least one thousand counts was recorded when determining background counts, i.e. a Standard Proportional Error of three per cent.

Finally the Standard Errors of nett count rates was calculated by: $S.E._{\cdot} = \sqrt{S.E._{\beta}^2 + S.E._{s}^2}$

β = background
s = sample

1.6. Radiological Safety Techniques.

All types of radiation produced by radioactive elements can damage living tissue. The extent to which special precautions are required in the handling of isotopes depends primarily on the amounts used and the relative radiotoxicity of the isotope. P^{32} is only a moderately toxic isotope along with isotopes such as Fe^{55} and Co^{60} and as such does not require many of the stringent precautions demanded when using isotopes such as Sr^{90} and other natural radioactive elements with a high mass number (Francis, Mulligan and Wormall, 1959).

The total amount of P^{32} used in this experiment was relatively minute (45 uc): this along with the fact that P^{32} has a short half life and a range of only a few centimetres in air, meant that normal laboratory areas could be used, providing a number of elementary precautions were taken. These were as follows:

1.6.1. Acid digestion was carried out in a fume cupboard.

1.6.2. All working surfaces involved in the transfer of acid digest samples were covered with thick absorbent acid resistant paper.

1.6.3. Solid and liquid waste contaminated with P^{32} was confined to one bin for separate disposal.

1.6.4. Plastic gloves were used when removing samples from the aquaria and other experiments.

1.6.5. The aquarium was covered with glass to prevent the loss of labelled organisms, e.g. midges, damsel flies, caddis flies and beetles.

1.6.6. The aquarium microcosm was raised off the bench surfaces with a number of two inch square wooden blocks. The whole area around the tank was enclosed within a rigid wood and plastic sheet frame. These precautions were taken in case it became necessary to isolate and repair any leaks occurring from the aquarium base.

1.6.7. All glassware was thoroughly washed before reuse to reduce the possibility of contaminating later samples in the processing procedure.

1.6.8. Acid digest and water samples contaminated by P^{32} were always transferred by using rubber bulb pipettes in order to eliminate any possible oral contact with P^{32}.

1.7. Problems of Method.

Aquarium communities are small but by no means simple. As in almost all synecological research, various problems arise in the sampling and separation of some community fractions and in the interpretation of what is taking place in the system at any one time. The Movement of P^{32} through such a system is highly complex and not all the major routes of transfer can be effectively sampled or separated out. Other factors affecting the complexity of movement include the inter-relating effects of almost all that happens in the aquarium microcosm.

The small size of the community and therefore the modifying effect sample removal has upon it will also complicate the picture. The effect of sample removal on the total P^{32} available in the system can be corrected for by subtracting the amount of P^{32} removed at each sample time from the total amount present before each sampling period. It was calculated that by the end of this experiment, about four per cent of the original P^{32} introduced had been removed by sampling procedures. Sample removal in this experiment had a less modifying effect on the community than in similar experiments carried out by Whittaker (1961). The effect of sample removal was insignificant in most cases, e.g. water, mud, sediment and millipore fraction, < 1% removed, sidewall community about 8%.

When determining the percentage distribution of P^{32} with time in the animal and plant fractions, one also must take

into account the effects of predation, herbivore grazing and mortality on these fractions in order to estimate the size of their P^{32} pools at any one time. The biomass of these fractions was known to a reasonable degree of accuracy at zero hours and also at 1200 hours and it has been assumed that the biomass of any one plant or animal species at a particular time lies at a point on a straight line drawn between the biomass estimates at 0 hours and 1200 hours, i.e. the populations were assumed to decrease in a straight line manner.

Total P^{32} distributions with time in the various community fractions have been interpreted on the basis of both the actual changing populations (estimated as above) and on a hypothetical constant one (that existing at zero hours) (Figs. 1 and 2, Tables 1 and 2).

Other problems are concerned with the effective separation of community fractions and the various modes of P^{32} uptake. The millipore fraction filtered out from the water was a complex mixture of algae, bacteria, protozoa, rotifers, organic and inorganic particles and colloids along with bacteria and other organisms attached to their surfaces. Sample examination under the microscope supported the assumption that algal cells predominated in terms of biomass though whether they were responsible for a major part of the P^{32} uptake into this fraction was another matter (Rigler, 1956). Such a mixture can be separated fairly efficiently (Whittaker, 1961), however sample volumes of two litres are required. This was considered

too large a volume (in terms of its effect on the community) to be usable and consequently finer separation was not carried out.

The organisms of the sidewall fraction formed a thick brown film over the glass substrate. Microscopic examination revealed that this consisted largely of algal and bacterial remains. Algae attached to the sidewalls included Lingbya sp., Oedogonium sp., Oscilatoria sp., along with large numbers of rod-like bacteria. It was virtually impossible in this experiment to assess the relative importance of these fractions in terms of P^{32} uptake into the organisms and onto the surface of the glass sidewalls.

The mud fraction probably had the most complex make up of all the aquarium fractions. It included the following fractions. Filamentous and unicellular algae, bacteria, diatoms, detritus particles, large pieces of decomposed organic material, fibres (mainly Potamogeton sp.,) large pieces of coal, coke and smelter ore, glass, sandy quartz and very fine coal dust particles. The total surface area of this heterogenous mixture certainly presented a larger surface area than that of the tank bottom (4,368 cm^2) on which it rested. Tracer data from the early hours of the experiment indicated that this community fraction took up about as much P^{32} from the water as the total glass sidewall fraction which had a surface area nearly three times greater.

This heterogenous mixture was difficult to sample because in the process of removing sample boxes from the bottom

of the aquarium some detritus and vegetable matter was invariably lost. It was probably this and other inaccuracies in mud sampling that accounts for the increasingly large P^{32} deficit towards the end of the experiment. The term deficit refers to P^{32} unaccountable for in the aquarium on the basis of sample data, e.g. forty-seven per cent at 1200 hours. It is assumed that a large part of this deficit was held in the mud, but remained undetected because of the rather crude sampling method (Figs. 6 and 7).

The sidewall community probably has an exaggerated importance in terms of P^{32} uptake, when compared to its importance in the field, because the large glass surface area in the tank provided an ideal and much enlarged habitat. In the natural state such a community is largely confined to the surfaces of aquatic plants around the pond edges.

On a number of occasions the mud samples were separated into two fractions and assayed separately. The figures obtained were variable but suggest that about twenty-five per cent of the mud P^{32} was concentrated onto the larger pieces of organic detritus by the end of the experiment. Another fraction that was impossible to assess accurately was the Chironomid, Oligochaete and Nematode fauna in the mud. It was originally thought that the mud was largely inert in terms of biological activity. Later observations of Chironomid emergence in the tank and microscopic analysis revealed the existence of these very small organisms.

Effective sampling of such small animals was,in the confines of this experiment, exceedingly difficult and not worthwhile. However, these organisms may well have provided an important food supply and P^{32} source for small carnivores and fish.

The movement of P^{32} through the aquarium fractions and organisms is a composite of several different kinds of uptake and loss which are not easily distinguished or estimated (Whittaker, 1961). Surface adsorption, as opposed to uptake via food and surface absorption, is a significant problem in a number of community fractions. For example, P^{32} uptake onto the glass sidewalls involves surface adsorption onto the glass surface and the living and non-living organisms and particles adhering to it. There is also active uptake by bacteria and algae, and the relative importance of these different processes cannot be estimated. A significant but variable part of the uptake by mud, sidewalls and the millipore fraction during the first hours is adsorptive. Thus the sidewall fraction uptake was made up of two components, one (adsorptive) reaching equilibrium in a few hours, and the other (absorptive) approaching equilibrium after fifty-three hours. (Fig.1). The major part of P^{32} uptake onto aquarium surfaces involves bacterial and microorganism films (Whipple 1901, Rigler 1956, Harris 1957, Whittaker 1961); these actively take up P^{32} and are often major loci of P^{32} concentration. Surface adsorption also occurred onto the surfaces of animals and plants in the initial hours of the experiment when the

P^{32} content dissolved in the water was high. Here again, this effect is largely due to the action of bacterial and other microorganism films (Harris 1957). As time passes and the P^{32} levels dissolved in the water fall off, the surface loadings of P^{32} concentrated by these organisms are increasingly reduced and diluted by exchange for P^{31} atoms. Examination of some of the activity density/time graphs, e.g. Fig.15 and 23, suggests that this may have occurred after about twenty to forty hours and result in the declining steepness of the uptake curves. This may imply that although the animal samples were thoroughly washed, a considerable fraction of more tightly bound surface P^{32} remained. Efficient removal of P^{32} can be achieved by washing in dilute nitric acid; this is impossible in a number of cases, e.g. leeches, snails, millipore papers and glass sidewalls. Also the millipore, sidewall, macrophytes and algal fractions have significant amounts of P^{32} which has entered chemical combinations on the cell surfaces and this is difficult to remove without affecting the more labile P^{32} in the cells (Gest and Kamen, 1948, Kamen and Spiegelman 1948, Goldberg et al 1951, Rice, 1953, Knauss and Porter, 1954). Thus for those organisms which are not easily separated from their skins, one is obliged to rely on the more or less deceptive values of P^{32} uptake recorded in the early hours of the experiment. In a number of cases (Limnaea pereger, Enallagma and Dytiscus larvae) P^{32} uptake into and onto the surface layers can be estimated. Uptake onto the shell surfaces

of Limnaea exceeded that into the soft body at one hour, but eventually levelled off at approximately twenty-four hours (Fig.12). P^{32} uptake into the soft body exceeded that of the shell after 7.5 hours, and peak activity densities were reached at about 120 hours. At this point the P^{32} load in the soft body exceeded that of the shell by about fourteen times, implying that little more than six per cent of the total organism load was held in the shell. Thus, if one combines the shell and body loads when assessing P^{32} uptake by Limnaea, one will obtain a deceptive picture of the "uptake" by the snail during the first few hours of the experiment.

In the early part of the experiment (up to 119 hours) a number of Dytiscus larvae caste their integuments; these were recovered and processed in the usual manner. The resulting activity densities for the two recorded points are plotted in Fig.18. Integument activity density was only marginally higher than that of the whole organism. The caste integuments made up about five per cent of the total body weight and held about two to three per cent of the total body P^{32} load. This suggests that only a very small proportion of the body P^{32} load was lost in the successive larval moults. Several Enallagma nymphs emerged during the first 144 hours of the experiment, these were removed and processed in the usual manner. The activity densities of the emerged nymphs was about a half to two-thirds less than the aquatic nymphs (Fig.23). This may suggest that up to fifty per cent of the P^{32} body load was lost on emergence in the cast off integument.

A more likely reason is that before emergence such forms reduce or stop feeding completely and therefore reduce their P^{32} intake, which in turn produces a lower level of P^{32} in emerged adults when compared to the level of loading found in the aquatic nymphs.

Physical adsorption no doubt affected the experiment in other ways. Adsorption of phosphate ions onto glassware and the plastic containers and apparatus used in transferring samples is well known (Parker and Barsom, 1970). This phenomenon can be controlled by using a non-radioactive carrier; however, extremely small amounts of such a phosphate carrier can support algal growth and blooms (Rodhe, 1948, Mackereth, 1953). Whittaker (1961) has observed that very small amounts of carrier phosphate sometimes produced accelerated algal growth in low phosphate aquaria, which in turn complicated the approach to equilibrium of the P^{32} content of the millipore and sidewall fractions. It was for this reason that no carrier was used when introducing the tracer into the aquarium.

Whittaker (1961) and Byers (1963) have pointed out that experiments with indoor aquaria are affected by the phenomenon of aquarium individuality attributable to minor variations in temperature, incident light and nutrient levels. These differences or contrasts between aquaria much exceeded expectation (Whittaker, 1961), e.g. there were up to three-fold differences in biomass and P^{32} content in some of his community fractions.

The time and facilities at hand only allowed the analysis

of P^{32} movement in one aquarium microcosm in this project. The absence of microcosm replicates does not imply that the results obtained were without real meaning or that valid conclusions on the movement of P^{32} cannot be drawn from the results. The observed uptake rates etc. are not put forward as absolute values which will be followed in the field, or in similar microcosms, but as a measure of the relative importance of the various steps in the movement of this element through the system. Thus while the picture based on the nine sets of sampling points supporting one another is not unconvincing, it must be remembered that the individual magnitudes given should be interpreted with the understanding that many of these values first have a gross or synthetic character and even as such lack real exactitude.

Biological variation in terms of the P^{32} content of community fractions and organisms presents another major problem when sampling from a small system with limited samoling material. Whittaker (1961) points out that biomass and P^{32} contents in the same community fraction can differ by up to two-fold in different samples from the same aquarium. He noted that duplicate water and millipore samples differed by small amounts in some cases during the early hours of the experiment. He attributed this to incomplete mixing and the rapid removal of P^{32} from water near the sidewall communities. For this reason water samples were removed from the same central point in the aquarium at about 5 cm. below the surface.

This value was taken as representative of the water body as a whole. Water samples removed from within the macrophyte mass at one end of the tank during the early hours of the experiment yielded filter water P^{32} values about fifteen per cent below those of the central samples. By about ninety-six hours the difference between the two samples was negligible. The lower value obtained earlier on was directly attributable to the rapid uptake of P^{32} from the water by the macrophyte ($\simeq$ 4.5 uc by one hour). As the P^{32} levels in the macrophyte approached equilibrium (53-168 hours) and the process of water mixing occurred in the macrophyte mass, the difference between the two sample points decreased.

Due to the effects of biological variation, the regularity of sample P^{32} contents (when plotted against time) depends largely on the number of individuals removed per sample. A balance had to be struck between the number of species individuals removed to produce reasonable activity density curves and the effect of excessive sample removed on the activity density of other species. This applies particularly to carnivores which rely on the herbivore population for food and their P^{32} loading. Table 10b and Figure 10a give the relevant information on biomass estimates and sample removal over the 1200 hours of the experiment. In Table 10b the column referring to the percentage biomass removed by 1200 hours includes those organisms removed at 1200 hours for the final sample and biomass estimates; in a number of instances these samples made up a major proportion of the total biomass removed in sampling.

Some fractions (Fig.15, 17, 22 and 23) exhibited conspicuously erratic P^{32} activity densities in the early hours of the experiment, when considering the general direction of the activity density graphs as a whole, i.e. 0 to 1200 hours. This was due to a number of variables, e.g. chance differences in food consumption, the degree of physical adsorption onto the animals surface, difference in feeding rates and behaviour. On the whole, as time passed, the biological variation between individuals of a species grew less. It was surprising that most of the graph points based on the activity densities of individuals (Dytiscus larvae and adults, Gasterosteus adults) produced reasonable activity density curves. Carnivores tended to produce more uniform and regular activity density curves than herbivores, even though larger numbers of individuals were used when sampling most herivore species. The larger carnivores, e.g. Dytiscus larvae and adults, Notonecta and Gasterosteus adults, were present at low densities because of their predaceous habits, and therefore provided only one or two individuals per sampling point. In an attempt to estimate the range of biological variation found in one species at one time, seventeen Hyphydrus ovatus individuals were removed at 1200 hours and their activity densities calculated and plotted up (Fig.25, Table 12). A clear trend of increasing activity density with increasing weight was found; this is discussed in a later section.

During the course of the experiment, no real climax or steady state of P^{32} concentration and movement was reached.

As the steady state was approached, the process of aging changes the community character and no equilibrium situation occurred.

PART TWO.

P^{32} UPTAKE AND LOSS EXPERIMENTS.

2. INTRODUCTION.

These two short experiments were concerned with the investigation of P^{32} uptake and, more importantly, its loss from two species of aquatic invertebrates, namely the Calanoid copepod Cypris sp. and the caddis fly larva Limnophilus stigma.

2.1. Limnophilus stigma.

Several hundred grams. (wet weight) of the filamentous alga Spirogyra were placed, along with thirty Limnophilus, in an aerated container holding 5.42 litres of pond water. On 23rd June 1972 at 12 noon, 1.25 uc (2.75×10^6 c.p.m.) of P^{32} were added. Three to five Limnophilus were removed at each of the following sampling times: 12.5, 56, 120, 168 and 240 hours, and processed in the usual manner. After ten days the remaining Limnophilus were removed, thoroughly washed in pond water and transferred to a similar container without any P^{32}. Two to four Limnophilus were removed at intervals of 24, 120, 216 and 360 hours after transfer and processed as before, in order to ascertain the rate of P^{32} loss when feeding off uncontaminated food in an uncontaminated environment. The pond water used was replaced every forty-eight hours in order to flush out any P^{32} excreted into the system by the Limnophilus. The results are set out in Table 26 and Figure 26.

2.2. Cypris sp.

A culture of several hundred Cypris along with their natural media and associated plankton was obtained from a rain butt near to the laboratory. One litre of culture water was placed in a large beaker; to this was added 0.5 uc (1.1×10^6 c.p.m.) of P^{32} at 2.00 p.m., 27th June 1972. At 2.00 p.m. on 11th July a sample of fifteen to

twenty individuals was removed and processed in the usual manner. At the same time the rest of the culture was removed from the contaminated medium with the aid of sintered glass funnels and transferred to an identical but uncontaminated culture medium. This procedure was repeated daily, i.e. the Cypris individuals were transferred to a fresh uncontaminated medium every day. Samples of up to sixty individuals were removed at intervals of 44.5, 144, 288 and 480 hours after the original transfer and processed in the usual manner to determine the rate of P^{32} loss from a given level of body load when feeding in uncontaminated media. Results are set out in Table 27 and Figure 27.

THEORY.

Rate Equations.

The theoretical basis underlying the calculation of rate values is given in Zilversmit et al (1943), Robertson (1957), Foster and Davis (1958), Whittaker (1961), Polikarpov (1966) and Polikarpov et al (1966).

The P^{32} contents of the processed samples were expressed in terms of counts per minute per gram dry weight, i.e. their activity densities. It was considered that these units were more easily comparable when assessing the graphs than the other unit of radioactivity, namely the Curie. One Curie produces 3.7×10^{10} counts per second, in this experiment the quantities of isotope used were measured in terms of microcuries (u.c.) where 1 uc. produces 2.22×10^{6} c.p.m.

The degree to which P^{32} was concentrated into organisms and community fractions was expressed in the form of concentration ratios (Table 9). A concentration ratio compares the activity density of an organism or community fraction with the activity density of water (from which the P^{32} is ultimately obtained) at the same point in time. Concentration ratios are given by:

$$C = \frac{A_o}{A_w}$$

A_o = A.D. of the organism or community fraction.

A_w = A.D. of the water.

All concentration ratios were based on the ratio of the activity density of an organism/community fraction in c.p.m./gm.(dry weight) to the activity density of water in c.p.m./ml. Two concentration ratios are given in Table 9, one is the concentration ratio at the maximum recorded activity density and the other is the maximum concentration ratio recorded during the time of the experiment.

The rates of P^{32} uptake by organisms and community fractions formed an important part of this work and were expressed in several ways. The gross uptake or transfer rates (r_1) express the proportion of P^{32} in one community fraction or species moving into another community fraction or species per unit time. For example, at zero hours 39.06 uc. were introduced into the aquarium water. After one hour sampling data showed that 20.30 uc. had moved into the millipore fraction suspended in the water. The transfer rate (r_1) of P^{32} from water to the millipore fraction is given by $\frac{20.30}{39.06}$ or .52 per hour.

That is, over half the P^{32} introduced moved into the millipore fraction in the first hour of the experiment. All of the r_1 values set out in Table 10a were based on data obtained during the early hours of the experiment, when the turnover loss of P^{32} was minimal. Transfer rates are useful when examining the movement of P^{32} in the ecosystem as a whole. Comparison of the relative rates of uptake of different species and community fractions can be expressed in terms of activity density. The activity uptake rate (r_2) measures the rate of increase of activity density in uc. of P^{32} taken up per hour per gram dry weight. Thus, if in the first hour 20.33 uc. of P^{32} moves into 2.20 grams dry weight of the millipore fraction, r_2 is given by $\frac{20.33}{2.20}$ or 9.23 uc/gram/hour.

The movement of P^{32} from water to organisms and back to water can be very rapid. In the case of the following community fractions, millipore, glass sidewalls, Callitriche and Spirogyra, the change of P^{32} content with time was affected almost from the beginning of the experiment by the return movement of P^{32} from these organisms to the water. It was for this reason that transfer rates and activity uptake rates were determined from the I^{32}

content of these organisms after one hour had elapsed.

Loss or turnover rates (r_4) via excretion i.e. metabolic P^{32}, were expressed as fractions of P^{32} leaving an organism or community fraction per hour. Turnover rates have been determined on the assumption that these must balance uptake rates at the maximum or equilibrium levels of P^{32} content.

Thus, when the rate of change of the activity density is zero,

$$\Delta A = 0$$

$$\text{then } r_2 = r_5 \quad \text{where } r_5 = A \times r_4$$

$$\therefore r_4 = \frac{r_5}{A}$$

r_5 = activity loss rate (uc/gram/hour)

A = activity density (uc/gram)

r_2 = activity uptake rate (uc/gram/hour).

The concept of turnover is a useful one for comparing the exchange rates between different components of an ecosystem. In terms of exchanges after equilibrium has been established, the turnover rate is the fraction of the total amount of a substance in a component which is released (or which enters) in a given time span. The turnover time is the reciprocal of this value, i.e. the time required to replace a quantity of substance equal to the total amount in the pool.

$$\text{Thus } r_4 = \frac{1}{T}$$

$$\therefore T = \frac{1}{r_4}$$

T = turnover time.

All r_1 and r_4 values calculated in this experiment were based on the original biomass estimates at spiking.

RESULTS.

PART ONE.

THE AQUARIUM MICROCOSM.

1. General Notes.

1.1. Rate Patterns.

The rate patterns set out in Figures 10b and 10c were determined from the initial period gross rates which were, despite their limitations, most directly subject to accurate measurement. These rate patterns express the rapid P^{32} transfer movements occurring during the first few hours of the experiment between the various living and non living community fractions involved. With the aid of certain rate equations and digital computer analysis (Whittaker, 1961, p.183) it is possible to determine the changing picture of rate values at various points in time as the experiment progresses, i.e. assess the effect of ageing and community development on P^{32} transfer movements between community fractions. In this particular experiment there was neither the time nor facilities to carry this out effectively and as a result only the rapid initial rate patterns are presented.

Gross uptake rates and transfer rates were compiled for every community fraction that was readily measurable. The accuracy of the gross uptake rate values depends largely upon the accuracy with which the trophic positions and feeding habits of the various species are known. The trophic positions were generally easily recognised, but the finer points of feeding relationships were in several cases difficult to observe, e.g. Sialis Larvae, Planaria, Leptocerus, Limnophilus stigma and Limnophilus xanthodes. The major sources of P^{32} in most cases were determined by repeated observations during the experimental period.

Although the major sources could be identified fairly accurately, the relative importance of the sources in contributing to the gross uptake rate of the species concerned was impossible to determine. For example, if a carnivore feeds off three major prey species there was no way (considering the limitations of this experiment) of determining their relative importance in the diet or as P^{32} sources. In view of these and other uncertainties no attempt was made to draw up a rate pattern diagram involving all the fractions and species sampled. Instead, a simplified diagram of P^{32} transfer between the major community fractions (water, plants, carnivores, herbivores, detritivores and mud) was drawn up (Fig.10b). Figure 10c sets out those rate patterns for which the feeding relationships were felt to be reasonably accurately known.

It must be noted that the r_1 values calculated for the herbivore and carnivore fractions in Fig.10b were derived by determining the uptake in uc/hr. of the whole fraction, i.e. all species of these groups and assuming that this was taken from the <u>total</u> P^{32} pool available in the preceding trophic layer. This produces a slightly different picture when compared to the one obtained by using the more specific food chain data.

There were a number of pathways which could not be measured directly or even estimated; these are included in Fig.10b, but no rate values were assigned to them, e.g. bacteria and fungi feeders in the mud, oligochaetes, nematodes and chironomids etc.

A number of community fractions, e.g. millipore, sidewalls, are composed of several types of organisms (bacteria, algae) and components (colloids, detritus, particles) each having different rate values and patterns but producing an overall rate pattern into the community fraction as a whole. In this sense, such rate patterns and values expressed for these fractions can be considered somewhat "synthetic". The following section refers to general notes on P^{32} sources and food chain observations:

The P^{32} dissolved in the aquarium water was the only source for the producer trophic levels (millipore fraction, sidewalls, Spirogyra and Callitriche) and the r_1 values (gross uptake or transfer rates) calculated for this level can be expected to have a good degree of accuracy.

The feeding habits and therefore the major P^{32} sources for the herbivore species were in most cases easily determined, both by observation and later analysis of the P^{32} activity density graphs. For instance, the three caddis species, Limnophilus stigma, Limnophilus xanthodes and Leptocerus sp. were observed to exhibit different behaviour patterns with regard to feeding. L.stigma was largely confined to moving amongst the bottom ooze and mud; this observation along with information from various texts labels the organism as a detritivore feeding off large pieces of organic detritus. The closely related L.xanthodes rarely appeared on the bottom mud and was invariably found feeding amongst the Callitriche stems or near the surface (it is a very active swimmer with a much lighter case than L.stigma). Thus on observation alone the two species appeared to exhibit clear cut feeding differences:

this difference shows up in the activity density graphs (Fig.15). For example, L.xanthodes had a much higher activity uptake rate (0.0188 uc/gram/hour as opposed to the 0.00175 uc/gram/hour of L.stigma) and reached a higher maximum activity density (0.60 uc/gram as against 0.45 uc/gram for L.stigma). L.xanthodes also reached its peak activity density earlier on (612 hour) than did L. stigma, (864 hour). This data supports the original observation, i.e. L.xanthodes was feeding off green plant tissue with a high activity density (0.25 uc/gram at 96 hours) and consequently has a faster uptake to higher levels than L.stigma which was feeding off dead material with a low activity density. Another related fact is the decline in activity density of L.xanthodes after 612 hours due to the declining P^{32} levels of its food Callitriche, while L.stigma's activity density carries on rising to the last measurement at 864 hours as an increasing proportion of the free P^{32} in the system moves downward in the form of dead tissue to the mud.

The remaining caddis species Leptocerus was observed either attached to the aquarium sides or moving over the mud. Their activity density curves were very similar to those of Limnophilus stigma despite the large variation in weight. This species was assumed to feed primarily on the sidewall fraction, though the form of the curve suggests that it may have been feeding off detritus rather than living plant tissue, i.e. the curve carries on increasing up to the last recorded measurement at 1200 hours (Fig.15).

The other major detritivore Corixa sp. feeds primarily on the nascent ooze produced mainly by the millipore and sidewall fractions. The gross uptake rate for this species was calculated on the assumption (not unjustified in view of the activity density curve shape) that it took its P^{32} directly from the settling dead plankton and bacteria of the millipore fraction, rather than from the "sediment".

The feeding habits and major P^{32} sources of several carnivore species, e.g. Dytiscus larvae and adults, Notonecta, Agabus and Gerris were fairly uniform and easily determined. Others, e.g. Erpobdella (leech), Sialis larvae, Polycelis (planarian) and Nepa, were harder to ascertain. Polycelis was never seen feeding but was found with regularity in empty Leptocerus cases or with partially eaten Leptocerids. It was probably a general scavenger (it is usually quoted as taking small invertebrates especially oligochaetes), though Reynoldson has pointed out that its diet varies considerably with the availability of food sources.

The gross uptake rates calculated for Gasterosteus adults and fry, Enallagma nymphs and Hyphydrus beetles were based on the results of feeding on the large Daphnia populations present in the first week of the experiment, though changes of diet probably occurred after these populations declined, e.g. Gasterosteus switched to bottom feeding.

1.2. Notes - Figure 10b.

Figure 10b illustrates the major pathways of P^{32} cycling in the aquarium microcosm and the transfer rates (r_1) between different community fractions. The decimal fractions represent the proportion of P^{32} in one community fraction moving into another community fraction per hour. The basic system is one of movement between the water, plants, herbivores, carnivores and between the sediment - detritus complex and the detritivores. There are return pathways to the dissolved state via direct exchange (plants) and excretion (animals), solid faeces is assumed to move downwards to the mud along with the products of death and decay. The P^{32} movement into the mud is complex, involving several pathways and processes, e.g. death and decay, faeces, uptake by the biological component from the water, and perhaps chelation of free P^{32} with the inorganic mud compounds. Other possible pathways are included, namely that of the algae-bacteria feeders present in the mud in unknown quantities and with unestimated r_1 and r_4 values (nematodes, oligochaetes chironomids).

The values given for the following fractions (plants, herbivores and carnivores) were calculated in the following manner. In general the r_1 values were calculated on the basis of figures obtained during the early hours of the experiment. P^{32} uptake via the plant fraction (millipore fraction), sidewall algae, Spirogyra and Callitriche) was based on the one hour figures. The P^{32} held by this pool after one hour was nearly eighty per cent of the spike introduced at zero hours, this gives an r_1 value of .79.

From this pool the next step moves into the herbivore population. Since the P^{32} content of the plant pool after one hour was known (31 uc), the r_1 value into the herbivore level could be calculated. To calculate the P^{32} moving into the fraction the r_2 values and biomass figures (for all the individual species) were multiplied together and summed. This figure when expressed as a proportion of the P^{32} held in the plant pool gives an r_1 value of .04. The r_1 value between the herbivore and carnivore level was calculated in the same way giving a value of .017. Included with the herbivore figures in this calculation were the detritivore figures, which obtained their P^{32} from the organic sediment-detritus complex.

Return rates for these fractions were calculated from the r_4 values given in Table 10a using the following, r_2 x biomass x r_4 = uc lost per hour from the pool. This calculation was carried out with each species and the results summed and expressed as a proportion of the total pool. The loss rates from the herbivore, carnivore and detritivore pools are given, along with the loss rate from the three fractions treated as one large pool (.28). Naturally, this figure will be closest to the largest fraction contributing to the pool, in this case it was the herbivore pool with a loss rate of .29.

The detritivore uptake from the sediment-detritus pool was calculated on the basis that Corixa was utilising a proportion of the total millipore and sidewall community,

and that Limnophilus stigma was utilising P^{32} held in the larger pieces of detritus, which was estimated at ten per cent of the P^{32} found in the mud layer.

The total P^{32} moving into the mud fraction during the experiment came from a variety of sources; the scheme set out in Fig.10b was arrived at on the basis of experimental observation and references in the literature on fresh water bodies and the relation of phosphate circulation to mud components.

1.2.1. Settling sediment - largely composed of dead millipore and sidewall fractions, along with the faeces of the herbivore, carnivore, and detritivore levels. This was a directly measurable quantity (Table 8, Figs.5 and 8) and its value was estimated at three points during the experiment. All of this quantity was assumed to move into the inorganic mud layer, i.e. the aquarium bottom, i.e. its r_1 value was equal to 1.

1.2.2. Biological uptake - via mud organisms from the filter water, e.g. by bacteria, algae, diatoms and fungii. There may well have been some combination of P^{32} into inorganic chelating complexes associated with this uptake in the early hours of the experiment. For this reason the path leading from the water (Fig.10b) to the algae - bacteria - fungii box of the mud layer also included a path to the inorganic mud fraction. However, it is thought that chelation played a minor role in this uptake and that most of it was biological.

1.2.3. Death and decay of animals and the larger plant species.

The contribution to the mud P^{32} pool via the sediment was estimated on the basis of the first available figures, i.e. 168 hours. This figure only provides an average rate of P^{32} sedimentation over the seven days. The r_2 value in Table 10a was calculated by extrapolating the sediment activity density curve (Fig.5) back to one hour. This value one would expect to be the peak activity density of the sediment, since its main component (millipore fraction) reaches its peak activity density values at one hour and then declines. Using the above sediment information the amount settling into the mud over the first 7.5 hours was estimated at .35 uc. At this time there were 5.44 uc of P^{32} held in the mud (includes the deficit figures). There thus remains 5.09 uc (5.44 - .35) in the mud which it is assumed arrived via biological and physical means (see 2 above). This gives a figure of about .67 uc/hour entering the mud by mainly biological processes. This was removed from the 39.06 uc pool at zero hours in the water thus the r_1 value can be calculated as

$$\frac{.67}{39.06} = .017.$$

The amount of P^{32} contributed to the sediment and mud by faeces during the first 7.5 hours will be negligible, since it takes several hours for digestion and also general P^{32} distribution through the animal populations, i.e. up the food chain.

The movement of P^{32} from the millipore fraction, sidewall fraction and faeces via the sediment to the mud was estimated in the following manner. In the first seven days 7.81 uc (17.34×10^6 c.p.m.) moved to the sediment and thence to the mud surface. This gives an average rate of .046 uc/hour over the whole period. This was removed from a pool estimated at 22.24 uc. at one hour, giving an estimated r_1 value of $.0021\left(\frac{.046}{22.24}\right)$ from the above pool to the sediment.

The process of P^{32} movement into the mud fraction via the death and decay of the plant species Callitriche and Spirogyra and the herbivores, carnivores and detritivores was estimated on the basis of data obtained during the first fifty-three hours of the experiment. During this period the P^{32} content of the mud plus the deficit increased by 9.76 uc, the sediment contributed an estimated 2.44 uc of this. This gives a figure of 7.32 uc which it is assumed was derived from the processes of biological uptake and the death and decay mentioned above. The amount taken up by biological processes during the period was estimated at 5.1 uc, it is assumed here that the P^{32} content of the bottom algae and bacteria had not changed apreciably since the estimate calculated at 7.5 hours. This is based on the fact that the sidewall and bottom algae fractions behave

in a similar manner, i.e. reached an early peak P^{32} content, which remained fairly constant for about one hundred hours (see Table 1 sidewall algae data). This fact was based upon Whittaker's results in his aquarium experiments. The figure given is not meant to be taken as absolutely accurate but only to give an indication of the right order of the value. Subtracting the two figures (7.32 - 5.1) gives an estimated 2.22 uc moving into the mud via death and decay in the first fifty-three hours. This gives an average rate of 0.04 uc/hour moving into the mud via this route. This was moving from a pool with a size of 10.84 uc at one hour, giving an r_1 value of .0037.

The data pertaining to movement into the mud as a whole (Table 10a) was based upon the data up to fifty-three hours. 10.3 uc had moved into the mud during this period giving an uptake rate of .194 uc/hour. This was removed from a pool of 39.06 uc at zero hours giving an estimated r_1 value of .005. The r_2 value was calculated at 1.6×10^{-5} uc/gram/hour, this was calculated on the basis of the mud weight of 12 kg. The maximum activity density was of course recorded at 1200 hours, giving a value of .003 uc/gram. Turnover rate was calculated as usual and a value of .0053 obtained, this gives a turnover time of 190 hours.

1.3. Biomass Pyramid.

The total estimated biomass at zero hours is set out in Figure 10a, along with the production pyramid. The top carnivores consisted of Dytiscus larvae and adults (.98 grams), the figure for primary carnivores (3.61 grams) included the Hydracarina (water mites), detritivores (2.32 grams) were represented by Corixa and Limnophilus stigma and the herbivore level (C_1) includes the remaining herbivores plus the estimated biomass of the Limnea and Planorbis (mollusc) eggs along with the Daphnia. Molluscan egg biomass was estimated as ten times the weight removed by sampling during the experiment. The producer level consisted of Callitriche (37.6 grams), Spirogyra (10.0 grams) plus the sidewall (.625 grams) and millipore fractions (2.2 grams), this gave a total producer biomass of 50.425 grams. To this figure was added the estimated weight of the bottom algae ($\underline{\Omega}$ .2 grams) giving a figure of 50.625 grams or 51.0 grams to the nearest whole number. Bottom algae biomass was estimated on the basis of its area, which was roughly a third that of the sidewall fraction, giving a biomass of one third of the sidewall fraction which would be roughly in the right order of values, though Whittaker's (1961) figures are two to three times larger. Lastly, many small damsel fly nymphs were present, but never sampled, their estimated biomass was approximately (.22 grams), this weight was added to that of the larger nymphs (Enallagma) which were sampled and it was assumed (for the purposes of

transfer rate computations) that the two sizes of nymph behaved in the same way towards P^{32}.

The main sources of error in this pyramid was that production increases between weighing and zero hours could not be estimated.

2. Tracer Results.

2.1. P^{32} distribution patterns between the community fractions of the aquarium microcosm.

Figures 1 and 2 illustrate the changing patterns of P^{32} distribution with time in the aquarium microcosm. A major feature of this pattern was the very rapid movements occurring in the first few hours (0-22) of the experiment. A very rapid movement of P^{32} from the water to the millipore fraction occurred in the first hour (Fig.3). Within one hour, fifty-two per cent of the total P^{32} introduced had been concentrated into this fraction. This type of rapid P^{32} movement into suspended plankton is a universal feature in most tracer studies both in the laboratory and field (Coffin et al 1947, Hayes et al, 1952, 1954, Rigler, 1956, Whittaker, 1961). Soon after the one hour peak was reached the P^{32} content of the millipore fraction began to decline (along with that of the filtered water) as P^{32} in the water began moving into other community fractions in increasing amounts, e.g. Callitriche, Spirogyra and the sidewall fraction. Although these fractions had lower activity uptake rates (r_2) than the millipore fraction, they ultimately removed a major proportion of the introduced P^{32} from the water after about fifty-three hours (forty-nine per cent). The P^{32} contents of these fractions were due to the compound result of rapid uptake and loss processes. So although the millipore fraction takes up P^{32} very rapidly, it also loses P^{32} at a considerable rate ($r_4 = 1$). The other plant fractions involved have lower uptake rates and also lower turnover loss rates, they therefore accumulate P^{32} slowly, but return it to the

water at an even slower rate, the nett result being a gradual accumulation of free P^{32} from the water and out of circulation. Since the millipore fraction was losing P^{32} almost as fast as it took it up, the accumulation processes of the other plants gradually removed an increasing proportion of the free P^{32} and this resulted in the declining P^{32} content of the millipore fraction and the filter water fraction, from one hour onwards.

The P^{32} content of the animal fraction approached its peak slightly later on at about seventy-two hours. This was to be expected since it will take some time for appreciable amounts of P^{32} to move from the water and up the food chain into the animal fraction.

When compared to the other community fractions, the P^{32} content of the mud increases slowly at first (one per cent at one hour) and then more rapidly (fourteen per cent at seven hours, twenty-two per cent at twenty-two hours, forty-eight per cent at seventy-two hours) until at about one hundred hours it holds over fifty per cent of the total P^{32} introduced into the system. The fundamental importance of mud as a phosphate "sink" in natural fresh water ecosystems was illustrated by the fact that at 1200 hours ninety-six per cent of the original P^{32} was held here.

After about one hundred hours the P^{32} contents of all community fractions except the mud, began to decline as various processes, i.e. grazing, death, decay and predation took effect.

The P^{32} content of the sediment began to decline after the first few hours of the experiment (Fig.5) presumably as the P^{32} content of its major P^{32} source (the millipore fraction) declined in activity, after the initial one hour peak. Although sediment activity density and its rate of sedimentation (grams/hour and c.p.m./hour) decreases as the experiment progresses (Table 8), the quantity of P^{32} accumulated in the mud by sedimentation processes increased steadily through the experiment, e.g. 7.9 uc after seven days, 16 uc after thirty-six days and 18.4 uc after fifty days. After fifty days about forty-six per cent of the total P^{32} introduced had accumulated in the mud via sedimentation processes and this accounted for over half the P^{32} found in the mud at the end of the experiment. These figures illustrate the importance of phosphorous sedimentation rates in aquatic systems as opposed to the other routes of uptake by bottom algae, bacteria, ionic combination, precipitation of inorganic solids and other sediment sources, e.g. "macro" sediment of shells, bodies, cases and larger vegetable debris.

The rates and routes of P^{32} movement through this system present a different picture to that provided by Whittaker's experiments (1961). Millipore fraction gross uptake rates (r_1) were two times higher than any value recorded by Whittaker in his experiments. His "plankton" also exhibited activity density peaks at a later time (12-24 hours). After twenty-four hours (in his oligotrophic aquaria) some thirty two per cent of the original P^{32} introduced remained

in the aqueous phase, as opposed to about two per cent in this experiment. These differences were primarily due to the presence of Callitriche and Spirogyra in the tank and draws attention to the importance of such fractions as loci of P^{32} concentration under certain field situations, an observation also noted by Coffin et al (1949) in their experiments in an acid bog lake in Nova Scotia.

While the gross uptake rates of the millipore fraction exceeded those of Whittaker's by a large margin, the activity uptake rate was considerably lower than in his aquaria, (about seven times). This is directly attributable to the higher biomass of the suspended fraction in this system (about twenty times higher than Whittaker's averages).

The sidewall fraction in this experiment contained a much smaller proportion of the total P^{32} available in the system when compared with Whittaker's results at peak activity densities. In Whittaker's tanks, the sidewall fractions held between twenty-five and fifty-five per cent of the total available P^{32} in the system at peak levels. In this experiment the sidewall fraction never held more than six per cent of the total P^{32} available.

The animal fraction held a maximum of fifteen per cent of the total P^{32} by ninety-four hours, this declined to about one per cent after 1200 hours.

The estimated P^{32} content of the bottom algae, bacteria and microorganisms was put at 5.09 uc at 7.5 hours. This exceeded the sidewall content of 1.92 uc by two and a half times, this suggests that the surface area available on the

mud was many times larger than the sidewall area. The percentage distributions of P^{32} in this aquarium with time, parallels a number of observations made in lake and pond P^{32} tracer studies (Einsele, 1946, Hutchinson and Bowen, 1947, 1950, Orr, 1947, Smith, 1948, Coffin et al, 1949, 1949).

2.1.1. The rapid departure of P^{32} from the water, this was at first very rapid and later on became increasingly slower until an approximate water/"solids" equilibrium of P^{32} transfer was reached. This removal of P^{32} was not a log curve of constant fractions removed per unit time, but the result of two way transfer and exchange between the water and solids as in the lake data analysed by Hayes et al (1952).

2.1.2. The manner of removal and transfer, i.e., from water to plants, thence to animals and finally via the sediment to the mud, also corresponded to the general pattern observed in lakes.

2.1.3. The importance of the large masses of macrophytes, found under certain field conditions, in the concentration and retention of P^{32} from the aqueous phase (Coffin et al, 1949).

2.1.4. The fixation of a major part of the P^{32} in the system in the largely inaccessible benthic regions and mud, its release in response to changing aquarium conditions (i.e. ageing) and its effects on community productivity.

Finally, Whittaker (1954) carried out a number of experiments in aquaria fertilised at different PO_4^- levels from .05 to .52 p.p.m. He found that the fastest and most effective removal of P^{32} from the water occurred in low phosphate aquaria, these also produced the highest concentration ratios of P^{32} found in any aquaria. The rate of P^{32} removal and the concentration ratios recorded in this experiment suggest (on the basis of Whittaker's observations) that this was an aquarium in which low levels of dissolved phosphates occurred. His observations suggest that the addition of PO_4^- to water bodies up to unnaturally high levels may provide a useful method of P^{32} radiocontamination control.

2.2. Activity Density Curves.

Most of the graphs exhibited a number of common features.

2.2.1. A straight line increase with time towards the equilibrium levels (for some reason this was more clearly shown by carnivore than herbivore species).

2.2.2. The rate of increase slowed down as the maximum activity density levels were approached and finally reached.

2.2.3. This was followed by a decline in activity density in response to the declining P^{32} contents of the food and water.

In a few cases, e.g. Enallagma and the three caddis larvae species, there was a marked convexity after about one hundred hours where the activity uptake rate declined to a lower but more uniform level. This may have been due to the effects of physical uptake during the early hours of the experiment, changes in the type of food taken or sample inaccuracy.

It was expected that the curves would exhibit an initial concavity in shape due to the time lag for P^{32} labelled tissue to pass up the food chains. This appears to have been obscured by the effects of physical uptake in the early hours of the experiment, producing a straight line activity density increase. The only species for which a marked concavity in the curve was recorded was the leech Erpobdella (Fig.24). P^{32} was first

detected in this species after 119 hours of exposure, a rather marked time lag, perhaps due to its location in the mud layers.

Notonecta individuals exhibited a surprisingly low peak activity density (.15 uc/gram) when one considers that they are usually regarded as one of the most predacious insect species found in fresh water ponds. The water mites (Hydracarina), sampled throughout the experiment, initially reached a peak activity density of .027 uc/gram after one hour (Fig.21). By about sixty hours all this P^{32} appears to have been lost and probably represents the P^{32} initially adsorbed onto the animals carapace and which was later exchanged back to the water for non radioactive P^{31} atoms as the aqueous levels of P^{32} declined. This group was regularly sampled in sizeable numbers (up to fifteen individuals) during the whole of the experiment, yet at no time after sixty hours were measurable amounts of P^{32} detected. This fact suggests that they were not feeding, since it was impossible for them to feed off a non-labelled food or substance in the tank, as all surfaces and organisms living or dead had P^{32} on or within themselves. Water mites are usually referred to in the literature as zooplankton predators, but would not appear to be on the basis of the results obtained in

this experiment. Either their normal food supply was not included in the tank (which seems doubtful), or the adults feed very infrequently if at all during the spring and summer. Davis and Cooper (1951) in their study on the effect of Hanford effluent upon the aquatic invertebrates of the Columbia River found a definite accumulation of P^{32} by water mites, which suggests that a suitable food supply was not available in the tank although zooplankton were present in varying amounts throughout the experiment. There was a definite contrast between the activity density curves of the two mollusc species Limnea pereger and Planorbis albus (Fig.12 and 16). Limnea achieved higher peak levels of activity density, .68 uc/gram (Body and Shell), as opposed to .32 uc/gram for Planorbis. Limnea's activity uptake rate was about two times higher (.0081 uc/gram/hour) than Planorbis (.0037 uc/gram/hour) and it also reached peak levels at an earlier time (Limnea 120 hours, Planorbis 200-250 hours). The activity density decline of Planorbis appeared to be faster than that of Limnea. Since Planorbis had a smaller loss rate (.011) than Limnea (.013), it would be expected (if both species were feeding on the same P^{32} sources) for Limnea to decline in activity density at a faster rate. Since Planorbis in fact declines at a slightly faster

rate, this fact along with the others mentioned, suggests that although the two species appeared to feed off the same food sources (Callitriche and the sidewall fraction), that they were probably feeding off different components in these fractions, e.g. Limnea may have been feeding off Callitriche tissue, while Planorbis might have been feeding on the attached periphyton layer. The sidewall fraction was known to consist of several parts, which take up P^{32} at different rates and achieve different activity density maximums at different times, and these two species may also have been feeding off different sidewall components. This illustrates the possible use of radiotracers in the separation of feeding niches and habits in two related species.

Finally, Gasterosteus (Stickleback) adults and fry exhibit a number of contrasts in their P^{32} uptake. The rates of uptake in the two forms were about the same (Fry .0197 uc/gram/hour, Adult .01836 uc/gram/hour). However, the adult activity density curve levels off at about fifty hours, while the fry carries on accumulating P^{32} until about 160 hours. The fry reached a peak activity density of 3.54 uc/gram as opposed to a value of 2.50 uc/gram for adults. A number of possible explanations for this are put forward below.

A switch in food supplies by the adult after fifty hours, or the fry were utilising more of the P^{32} taken up in tissue building and holding this P^{32} for longer periods. Adult growth was obviously slower, along with its metabolic rate and less assimilated P^{32} may be laid down and held in new tissues, i.e. P^{32} uptake rates are the same, but the fry holds more of this for a longer period in the body. The adult may also have a larger pool of labile PO_4^- which is excreted relatively quickly.

2.3. Rate Values.

The highest activity uptake rates (r_2 values) occurred in the following fractions and in the organisms feeding directly off them (Table 10a). Millipore fraction (9.23 uc/gram/hour), sidewall fraction (1.14 uc/gram/hour), Spirogyra (.5207 uc/gram/hour), Callitriche (.1186 uc/gram/hour), Daphnia (via millipore fraction) (.3909 uc/gram/hour), Rana tadpoles (via the sidewall fraction and Callitriche)(.7704 uc/gram/hour), Gasterosteus adults (via Daphnia)(.0184 uc/gram/hour) and fry (.0197 uc/gram/hour). Carnivores generally had lower activity uptake rates than most herbivores, their loss rates, however, were much smaller and consequently their turnover times were longer. As a result the time it took them to reach peak activity densities was on average only slightly longer than for the herbivore species. In most cases carnivore P^{32} contents declined at a slower rate due to their lower excretion rates and longer turnover times (Figs. 11-24). There seemed to be no clear cut relationship correlating the magnitude of the uptake rate, the maximum activity density achieved, the turnover rate and the turnover time to size as Whittaker (1961) has stated. Activity uptake rates appeared to depend primarily on the P^{32} content of the food eaten, i.e. on food chain or trophic level relationships. In one case involving three beetle species, Whittaker's relationship holds. In order of increasing size these were Hyphydrus, Agabus and Dytiscus adults. The following points hold:

2.3.1. As the individual size increases its r_2 value decreases.

2.3.2. The turnover loss rate r_4 increases with decreasing individual size, i.e. smaller species lose their P^{32} at a faster rate.

2.3.3. As a result of (2) the turnover time increases with species size.

2.3.4. The maximum activity density decreases as individual size increases.

Other closely related species, e.g. the caddis larvae Limnophilus stigma, Limnophilus xanthodes and Leptocerus, showed no such size dependent relationships. Whittaker (1961) in his artificial outdoor pond experiment implied that there was an inverse relationship of the type stated for the three beetle species which held for all aquatic invertebrates. He says this relationship results from the faster growth and higher metabolic turnover in smaller organisms along with trophic level relationships, i.e. P^{32} content of food sources. His conclusions were based on data for seven species of consumers (two herbivores and five carnivores) and in view of the small number of species he examined, along with the results of this experiment it is hardly feasible to make such generalisations about uptake and size relationships until more detailed work is carried out under natural field conditions.

2.4. Rate Patterns.

The complex patterns of movements and rates of movement of P^{32} through the various fractions of the aquarium microcosm can be expressed in the form of rate patterns. These are the primary factors determining the relative concentrations of P^{32} in the various community fractions through time. P^{32} movements in the aquarium microcosm involved more than one given set of rate values in operation during the period of the experiment. Rate patterns should, therefore, be understood to have a temporary meaning and that the rate pattern at a given time cannot be used as a basis for the prediction of P^{32} distribution over a longer period. The processes of aquarium ageing, variations in the relative importance of adsorption processes during the earlier stages and the changing processes of absorption and ingestion combine to produce a shifting complex picture of rate patterns as time passes.

The major routes of P^{32} cycling and the initial transfer rates calculated for the early hours of the experiment are presented in Figure 10b.

The most noticeable features were:

2.4.1. High uptake and loss rates of the plant fraction, especially the millipore fraction (Table 10a).

2.4.2. The increasingly smaller proportion of P^{32} removed from preceding trophic levels by the herbivore and carnivore consumer levels.

2.4.3. The apparently greater loss of P^{32} from the herbivore level via excretion (.29) than via predation (.017), this fact was also noted by Whittaker (1961).

2.4.4. The lower carnivore excretion rates, implying that they retained their P^{32} body loads for a longer period than most herbivores.

2.4.5. The slower processes of P^{32} accumulation in the mud via sedimentation (.0021), death and decay (.0037) and biological routes (.017).

The return rates (r_4) to water in some fractions was much larger than the uptake rate (r_1) e.g. herbivores and detritivores. This is quite possible since the uptake rate was calculated on the proportional uptake from a very much larger P^{32} pool.

Figure 10c sets out a number of individual rate patterns which were known reasonably accurately. Both the gross uptake rates and turnover rates are given. The plant data suggests that the filter water turnover time was 1.21 hours, i.e. all the P^{32} originally introduced was passing through the plant population within this time. Naturally this was an initial very rapid reaction which would slow down as P^{32} became more evenly distributed in the system.

Plant uptake rates grew slower as cellular complexity and organisation increased, e.g. millipore

fraction (r_2 = 9.23 uc/gram/hour), sidewall fraction (r_2 = 1.14 uc/gram/hour), Spirogyra (r_2 = .5207 uc/gram/hour) and the macrophyte Callitriche (r_2 = .1186 uc/gram/hour). The turnover rates also followed a similar pattern. The remaining points of obvious interest have all been mentioned in the previous paragraphs.

2.5. Concentration Ratios.

The figures given in Table 9 do not have an absolute meaning as such, but can be used in a comparative sense. For under the conditions of this experiment no real steady state of P^{32} content was reached. The maximum activity density values represent inflexion points (where P^{32} loss exceeds P^{32} uptake) and not stable equilibria. The activity densities at these points were the result of P^{32} uptake in relation to P^{32} content of the food and water at the same time, and also in relation to the changing P^{32} contents of these during the preceding periods. Thus, maximum activity densities were reached at different times for different species and, therefore, at different water activity densities, this produced concentration ratios which are not strictly comparable in quantitative terms. Nevertheless the figures obtained illustrate some important general points.

2.5.1. The degree to which phosphorous compounds (and radioactive isotopes) are concentrated from the water into living organic matter.

This concentration process when linked with the very rapid disappearance of P^{32} from the dissolved state suggests strongly:

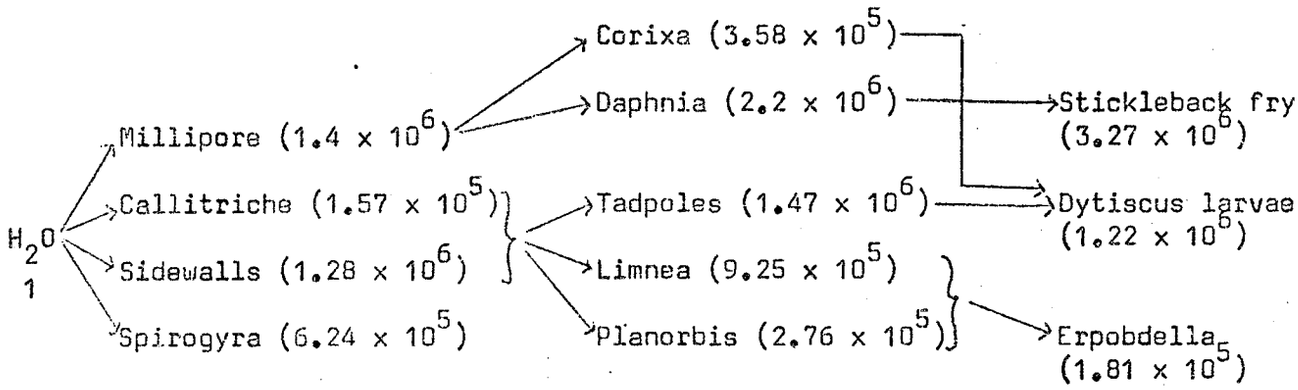
i. That there was a shortage of phosphorous in the aquarium, i.e. it was nutrient poor.

ii. Illustrates the importance of phosphorous to living organisms as a basic component of all cell energy transfer processes.

iii. And the fact that some aquatic organisms have evolved methods of rapid concentration and storage from low phosphorous environments, e.g. millipore fraction, sidewall algae, and other multicellular plants.

2.5.2. Phosphorous is generally found at low concentrations in aquatic media, this fact in connection with the high demand for it (by living organisms) has important consequences upon the behaviour of P^{32} effluents released into aquatic environments from nuclear power installations. High concentrations of P^{32} released, in conjunction with the high demand for phosphorous compounds by living organisms, can under certain circumstances combine to produce measurable effects upon the viability of aquatic ecosystems.

2.5.3. That concentration ratios tend to increase as one moves up the food chain (see below)



2.6. Biological Variation.

Biological variation in the P^{32} content (activity densities) of species individuals was observed on a number of occasions. At 168 hours two Gasterosteus adults removed at the same time had respectively activity densities of 1.16 uc and .94 uc, a variation of about ten per cent. Two sets of Gasterosteus fry removed at 363 hours contained 2.95 uc and 2.76 uc, a variation of about seven per cent. Two Dytiscus adults removed at 1200 hours showed a variation in P^{32} content of about two hundred per cent; this large difference was probably connected with the fact that the larger individual was three times the weight of the smaller individual.

Herbivore biological variation was in the case of Limnea pereger bodies far more erratic than carnivores. Some figures given below illustrate this:

1 hour, .0014 uc to .0024 uc (180% variation).
8 hours, .019 uc to .09 uc (450% variation).
168 hours, .3 uc to 1.2 uc (400% variation).

Seventeen Hyphydrus ovatus, processed separately at 1200 hours showed a surprisingly wide range in activity density. Activity densities varied from .05 uc to .45 uc per gram, i.e. a nine times variation. There appeared to be a definite trend of increasing activity density with increasing weight and size (Fig.25). The data suggests the existence of two activity density/weight classes, where individuals above .0051 grams have high activity densities above .27 uc per gram, and those below .0051 grams activity densities below .27 uc per gram.

Whittaker (1961) points out that smaller organisms and individuals show faster uptake rates (r_2) and achieve higher activity densities in a shorter time than larger individuals. If this holds true, why was there a definite reversal of this trend in the case of Hyphydrus ovatus? A number of suggestions are put forward below.

2.6.1. Smaller individuals may take up P^{32} faster and reach higher activity densities after a short time period, but they may also have faster rates of P^{32} loss than larger individuals. As a result, after 1200 hours in a medium contaminated with P^{32} the smaller specimens would have lost most of their P^{32} producing low activity density values, while larger individuals (with slower rates of P^{32} loss) would retain more and produce the curve in Fig.25.

2.6.2. Smaller individuals may not have loss rates intrinsically faster than larger individuals (when compared to their uptake rate). However, since smaller specimens reach the equilibrium level sooner, they are declining from peak levels for a longer period than the larger individuals which reach peak activity density values later on. This situation could eventually produce a curve similar to that in Fig.25.

2.6.3. Smaller individuals may have lower activity densities resulting from some "competition" effect for food with larger individuals.

2.6.4. Whittaker's generalisation may not hold true under all conditions, especially in the unnatural conditions found in laboratories and artificial systems created outdoors. As of this time little work with P^{32} tracers has been carried out under natural conditions into the question of uptake and loss rates of individual aquatic invertebrate species. Odum (1961) has pointed out that the biological half life for heterotherms is not a constant value, as their activity rates vary widely. He also noted that laboratory and field values for excretion rates varied widely in heterotherms, and suggests that some other factor (apart from varying activity rates) such as dietary changes may be responsible.

2.7. Production Estimates.

Under certain conditions, one can estimate primary and secondary productivity via a disappearance of raw materials approach, utilising radioactive tracers such as P^{32}. A major problem of this approach is that many purely physical and chemical factors influence the rate of nuclide exchange, and as a result it has proved difficult to distinguish between biological and physico-chemical uptake.

The data derived from the experiment can be used to give a rough estimate of the productivity of the system (Whittaker, 1961).

It is assumed in this method (Odum et al, 1958) that the gross uptake rates (r_1) into the various community fractions expresses productivity. These rate values are assumed to reflect primarily the intake and utilization of phosphorous compounds in metabolism and the synthesis of organic compounds in growth and to be significantly correlated with productivity. It is further assumed that to the rate for one trophic level should be added the rates for all higher trophic levels dependent on it, (Fig. 10a). For example, the rate into the producer level from the water was 78.65 (all r_1 values (transfer rates) in Fig.10a are multiplied by 100 for convenience), from the producer to herbivore level 23.36, from the herbivore level to the first carnivore level 3.48, and from here to the top carnivores .537. The total (to the nearest whole number) was 107. The figure for the herbivore level (calculated as above) was 27, and for the two carnivore levels

was 4.0 and 0.5 respectively.

To the production estimated correspond efficiencies of twenty-five per cent for the second and fourteen per cent for the third and fourth levels. These values can be compared with other figures found in the literature.

	Whittaker (1961)		Lindemann (1942) *
	Outdoor Pond.	Cedar Bog Lake	Lake Mendota
Level 2	11%	13.3%	8.7%
Level 3	4.5%	22.3%	5.5%
Level 4	-	37.5%	13.0%

The efficiency of the herbivore level in this experiment would appear to be up to two times the values recorded in the wild (Lindemann, 1942). This is not too surprising considering the temporary nature of the community, the effects of P^{32} adsorbtion during the early hours of the experiment (upon which figures the production values are calculated), the uncertainty of a number of food chain relationships and also the P^{32} uptake by bottom algae.

Recent work by Odum and Golley (1963) suggests in fact that the measurement of nuclide loss rates will prove to be ecologically more interesting than the uptake rates.

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* Note: Lindemann's figures are based on energetics studies not involving radioactive tracers.

2.8. Aquarium Ageing.

The process of aquarium ageing is reflected in the overall picture of P^{32} movements, i.e. a number of processes remove P^{32} from the water, binding it into biological material which ultimately moves downwards (via excretion, death and decay) through the system into the mud via several routes. Figures 8 and 9 illustrate some of the measurable changes detected in the aquarium community during the experimental period. Figure 8 shows the increasing amount of sediment accumulating (along with P^{32}) in the mud. After 1200 hours an estimated 104 grams and 18.34 uc of P^{32} had passed via the sediment into the mud, though it is not known how much of this sediment and P^{32} was recirculated back up from the mud, or if extensive recirculation in fact occurred.

Figure 9 records biomass changes occurring in the millipore and sidewall fractions in the aquarium. Sidewall biomass reached an initial peak about six days after the addition of P^{32}, from six to about thirty six days, the biomass declined gradually. Between day thirty-six and fifty, a second biomass peak developed reaching a recorded maximum of 3.2 grams sidewall biomass.

These biomass changes fit in with general observations made during the development of the aquarium community. The following pattern was observed:

2.8.1. The gradual accumulation of a brown film over the glass surfaces of the tank, this reached its maximum density at about ten days.

2.8.2. The progressive deterioration of this layer between ten and forty days.

2.8.3. At forty days, about seventy per cent of the aquarium glass was almost completely free of the original film.

2.8.4. During the next ten days there was a rapid build-up of a completely different film over the aquarium glass. This comprised mainly of a layer of green algae not previously noted in such large quantities before. Paralleling this new development was the production of large mats of blue-green algae on the mud surfaces, here again no such noticeable layer had been observed previously during the time the tank was set up.

These events indicate that some fundamental change was occurring in the system during the last two weeks of the experiment. It is interesting to note that the P^{32} content of the filter water rises from .189 uc at 864 hours to .22 uc at 1200 hours. The following scheme is put forward as a possible explanation:

Phosphate compounds bound up to non-living mud components were being gradually released into the water as anaerobiosis occurred in the mud layers. The release of nutrients from the bound pool in the mud was indicated by the sudden appearance of a prolific algal growth over the now nutrient rich mud layers.

Nutrient release and circulation produces the appearance of a new sidewall community in a relatively short space of time. During this

period the total P^{32} content of the sidewall fraction increased from .55 uc to .73 uc, i.e. by about thirty per cent.

The preceding observations indicate changing nutrient conditions were arising towards the end of the experiment and that these changes were in turn producing changes in the structure of a number of community fractions possibly due to the release of phosphates in the mud by anaerobic processes.

Over the same period there was no comparable increase in the biomass of the millipore fraction or of its total P^{32} content. This might imply that processes other than the ones above were at work, or perhaps more likely that the weighing techniques at these low levels of biomass was too innaccurate to detect any biomass fluctuations. The biomass of this fraction appeared to decline through the experiment, perhaps due to the nutrient shortage and the competitive effects of the macrophyte Callitriche and Spirogyra on P^{32} availability and balance in the water.

The sidewall biomass decrease from day six to thirty-six was probably the composite result of grazing, bacterial action, and a lack of essential nutrients in the water.

Note: The term sidewall biomass refers to all the organisms on the sidewalls; these consisted mainly of algae, bacteria and varying amounts of decayed organic matter (animals excluded).

PART TWO.

P^{32} UPTAKE AND LOSS EXPERIMENTS.

1. CYPRIS.

This experiment illustrates a number of points generally observed in most radioactive isotope uptake and loss experiments. Within a few minutes of the introduction of the P^{32} spike, the green unicellular algae in the culture had concentrated into their tissue about ninety-seven per cent of the introduced isotope atoms. Equilibrium of P^{32} movement between the liquid and solid phase (algae) occurred after a few hours with a 99:1 ratio of P^{32} in plankton to P^{32} in water. The Cypris concentrated P^{32} to a high level primarily by the food chain water - plankton - Cypris. After fourteen days the P^{32} content of Cypris had reached an activity density of 6.81 uc/gram. The activity density of the culture water (filtered) at this point was 1.5 counts/ml ($\triangleq$ .68 x 10^{-6} uc/ml); this implies a P^{32} concentration ratio of 1.0 x 10^{7} for Cypris. This was the highest concentration ratio recorded by any organism in all the experiments carried out. The above figure bears out the general conclusion that concentration ratios depend primarily on the P^{32} concentration in the food source.

The activity density of Cypris at fourteen days implies an averaged activity uptake rate of .02 uc/gram/hour. The P^{32} loss graph (Fig.27) shows a decrease in activity density from 6.81 uc/gram to 2.8 uc/gram after 144 hours in an uncontaminated solution. This gives an averaged activity loss rate of .028 uc/gram/hour. From 144 to 480 hours, about 1.4 uc/gram were lost giving a slower average activity loss rate of .0041 uc/gram/hour during this period, i.e. just over five times slower than during the first 144 hour period.

The presence of two distinct activity loss rates in the graph (Fig.27) is taken to imply the existence of at least two distinct metabolic micropools of P^{32} within the organism. These two pools appear to exhibit different turnover and loss rates which produce the two different curve values and slopes in Figure 27. The rapidly lost component (0-144 hours) may represent unassimilated P^{32} being discharged as faeces, or a labile metabolic pool with a quick turnover and loss via excretory methods. The second pool with a lower rate of loss (144 - 480 hours) probably represents P^{32} assimilated into the body structure, e.g. valve or shell and lost at a much slower rate which only becomes apparent after most of the labile pool has been expelled. The importance of loss rates and their applications is discussed by Odum and Golley (1963) and Southwood (p.361-363). Odum in his work on Zn^{65} uptake in aquatic invertebrates also suggested the existence of two discrete isotope pools.

(1) A non assimilated pool which is lost rapidly at a rate dependent on the "tag" level and the physical-chemical nature of the environment.

(2) An assimilated pool distributed through the tissue which is excreted at a slower rate and at a rate more directly related to metabolic processes.

2. LIMNOPHILUS STIGMA.

The results of this experiment show a similar pattern of uptake and loss (Fig.26). The rapid initial uptake and loss implies that a large proportion of P^{32} taken in was not assimilated. Within twenty-four hours, about half the ingested P^{32} was lost in the uncontaminated solution. Cypris on the other hand had only lost six per cent after twenty-four hours in the uncontaminated solution. It is suggested that a large proportion of the rapidly lost P^{32} was externally adhering to the caddis larva through the agency of bacteria and possibly fungii. This pool would rapidly come into an equilibrium of P^{32} transfer with water, and since the P^{32} atoms would be rapidly diluted (by exchange for non radioactive P^{31} atoms) and dispersed in the uncontaminated solution, a rapid loss of P^{32} from the exterior would occur. The importance of surface adsorbed/absorbed P^{32} by living and non living agencies is worth further investigation as other short experiments (not reported in this thesis) suggest that much of what was referred to as "physical uptake" in the past by various authors, may in fact be surface uptake.

SUMMARY

INTRODUCTION AND METHODS

Radioactive isotopes are a comparatively new tool in the field of environmental research. Phosphorous is an important macronutrient in aquatic ecosystmes and P^{32} can assume an important part of the aquatic contamination produced by certain types of reactor effluents. This study was carried out to illustrate the major paths of phosphorous cycling in an aquatic laboratory ecosystem and also to illustrate some of the mechanisms by which isotopes such as P^{32} can pose a hazard to aquatic life forms. Other facets of the study include the use of radiotracer information for the purposes of production estimates and in determining individual uptake and loss processes.

The main experiment was carried out in a 120 litre aqarium stocked with a representative cross section of pond life, water and mud. The system was allowed to settle down for three weeks before a "spike" of 39.06 uc of P^{32} was added. Samples were removed at intervals until the experiment was terminated 50 days later. The following fractions were sampled regularly (water, filtered water, suspended matter, mud, sidewall community, two plant species, nine herbivore species, twelve carnivore species and occasional long term sediment samples). Samples were generally taken at the following times, 1, 7.5, 22, 53, 168, 363, 555, 864 and 1200 hours after the introduction of the spike. All samples requiring drying were dried to constant weight at $70^{o}C$, weighed, dissolved in a hydrogen peroxide/nitric acid mixture and their radioactivity measured on a liquid counter and scaler apparatus. The results obtained were corrected for background interference, half life decay, instrument variations and efficiency and converted into a counts per gram dry weight figure. In all cases a standard error was calculated for the count rate. The results were processed further to produce a number of different value concepts. The most important were the activity density, the activity uptake rate, the gross uptake rate, the activity loss rate and the concentration rates.

A number of problems of method produced certain limitations within this type of experimental framework. The most important included the smallness of the system

and the shortage of sampling material, the difficulty of separating out and individually analysing the planktonic, sidewall and mud community fractions, the difficulty of discriminating between and assessing the relative importance of absorbtive and adsorbtive uptake by the various fractions in the system and finally the effect of individual biological variation in the uptake of P^{32}.

The remaining two experiments involved the P^{32} uptake and its later loss in an uncontaminated solution by Limnophilus stigma (caddis) and Cypris (copepod) populations.

Rate pattern diagrams for the main experiment were drawn up (Figs. 10b and 10c) using the initial period gross uptake rates. These patterns express the rapid transfer movements of P^{32} during the first few hours of the experiment. A number of these rates were estimated, an account of the methods used is given in the relevant section.

Finally, the living biomass was estimated just prior to zero hours, giving estimates of 51 grams for producers, 9.63 grams for herbivores, 2.32 grams for detritivores, 3.61 grams for primary carnivores and 0.98 grams for the top carnivores.

RESULTS

A major feature of the general isotope pattern was the very rapid movements of P^{32} from the water in the first few hours of the experiment. Within one hour, 52% of the total P^{32} introduced was concentrated into the millipore biomass, and 82% into producers as a whole. This is a universal feature of most tracer studies in the laboratory and field situation. The millipore figures imply that after a few hours there was little P^{32} in the tank that had not passed through the plankton. By this time little of the P^{32} would have been in the form of PO_4^{---} ions which are largely unavailable to phytoplanktonic algae (Chu 1946, Lund 1950). After 53 hours, the other producer fractions (Callitriche, Spirogyra and sidewall fractions) held 49% of the introduced P^{32}. After the initial one hour peak of the millipore filter fraction, the other producers (with slower r_2 rates) acted like a sponge accumulating, and more importantly, retaining P^{32}. Animal P^{32} content (as a whole) reached a peak at 72 hours after P^{32} had moved up the food chain. Mud P^{32} content increased very slowly, 1% at 1 hour, 14% at 7 hours, 48% at 72 hours, after 100 hours over half the original P^{32} had ended up in the mud "sink". After 100 hours, the P^{32} content of all fractions except the mud began to decline as various processes such as grazing, death, decay, predation and sedimentation took effect. Sediment P^{32} content began to decline after a few hours, its major P^{32} source was the millipore fraction which soon declined in P^{32} content. The rate of sedimentation (i.e. weight) also decreased as the experiment advanced. The quantity of P^{32} sedimented increased with time, e.g. 7.9 uc (168 hours), 16 uc (36 days), 18.4 uc (50 days), after 50 days about 46% of the total P^{32} introduced had accumulated via the sediment to the mud, showing the importance of sediment routes in aquatic systems. Rates of P^{32} movement through the system differed from Whittakers experiments, e.g. millipore r_1 values were two times higher than in any of Whittakers experiments, his plankton exhibit peaks at a later time (12-24 hours), after 24 hours in an oligotrophic aquaria 32% of the original P^{32} remains in the water compared to 2% in this experiment. This was primarily due to the absence of a macrophyte "sponge" in

Whittakers experiments emphasising their importance, an observation noted by Coffin et al 1949. In Whittakers experiments r_2 values were much higher (up to seven times) in the plankton, the result of a low biomass and absence of macrophytes. Whittakers sidewall fraction held far more P^{32} at peak values (25 - 55%), as opposed to a maximum of 6% in this experiment, of the total P^{32} available. The animal fraction held a maximum of 15% of the total P^{32} available at 94 hours, declining to 1% after 1200 hours. Bottom algae and bacteria held an estimated 5.01 uc at 7.5 hours in this experiment.

The basic patterns of P^{32} distribution are summarised below:

1. A rapid departure from the aqueous state into the phytoplankton and macrophytes.
2. The gradual accumulation of most of the P^{32} into macrophytes.
3. A later peak of P^{32} in the animal fraction as it moves up the food chain.
4. A gradual decline from peak values in all fractions.
5. The increasing accumulation of P^{32} in the mud, via sediment, death and biological uptake, until the majority of P^{32} rests in this largely inaccessible area.

Most activity density graphs showed the following common features. A straight line increase towards equilibrium levels, as this level was approached and passed the rates of increase declined in response to the declining P^{32} content of the food and water as P^{32} moved in increasingly larger amounts into the mud. The effects of surface uptake were most noticeable in herbivores in the early hours by the presence of a marked convexity in some curves. Other points of interest include the low uptake rate and low peak activity density level of Notonecta, usually considered to be the most rapacious of aquatic invertebrate predators. Water mites showed a surprising lack of P^{32} uptake after the initial surface adsorbtion peak had declined to unmeasurable levels, this implied no feeding occurred through the 50 days of the experiments, although food was available. Other curves, e.g. Limnea pereger and Planorbis albus, exhibit some possible uses of P^{32} in niche separation of food sources.

The largest rate values occurred in plants (millipore 9.23 uc/gram/hour, Spirogyra 0.5207 uc/gram/hour, Callitriche 0.1186 uc/gram/hour, sidewalls 1.14 uc/gram/hour) and those animal species feeding on them (Daphnia 0.39 uc/gram/hour, Rana tadpoles 0.7704 uc/gram/hour). Carnivores generally exhibited lower activity uptake rates than herbivores, however, their loss rates were lower and therefore their turnover times longer, and as a result, the time taken by carnivores to reach peak levels was only slightly longer than for herbivores. Unlike Whittakers experiments, there seemed to be no clear cut relationship correlating the magnitude of the uptake rates, maximum activity density values, turnover rates and turnover times to animal size, though this was exhibited by three coleopteran species. Activity uptake rates appeared to be primarily dependent on the type of food source utilized and its P^{32} content.

The major routes of P^{32} cycling and the initial transfer rates for the early hours of the experiment were calculated and set out (Fig. 10b). Noticeable features included the high uptake and loss rates by plants, especially the largely phytoplanktonic millipore fraction, the increasingly smaller proportion of P^{32} removed from preceding trophic levels as one moves up the food chain, the higher P^{32} loss rate of herbivores as against carnivores and finally the slow processes of P^{32} accumulation in the mud via biological (0.017), sedimentary (0.0021) and death and decay routes (0.0037).

The concentration ratios observed were not strictly comparable in quantitative terms since no steady state of P^{32} content was reached during the experiment. However, the results obtained illustrate certain important points, e.g. the degree to which phosphorous compounds, and therefore P^{32}, is concentrated from the aquatic environment into living tissues, the importance of phosphates to life, its relative scarcity in fresh water ecosystems and the existence of methods of rapid uptake and storage evolved by some organisms (plants) to cope with this. Lastly, as one moves up the food chain, concentration ratios tended to increase in magnitude.

Biological variation between individuals of a given species with regard to P^{32} contents was observed in a number of cases, this was due to natural differences in feeding rates, size, behaviour and other related factors. A detailed analysis of this variation was carried out at 1200 hours on 17 Hyphydrus ovatus individuals. Activity densities varied by 900% (0.05 uc/gram - 0.45 uc/gram), at this point in time there was a definite trend towards an increasing activity density with increasing weight. The data suggests the possible existence of two major activity density/weight classes above and below 0.0051 grams (Fig. 25).

A production estimate was made along the lines of a disappearance of raw materials approach using P^{32} gross uptake data, i.e. r_1 values. Comparisons with two sets of data by Lindemann (1942) and Whittaker (1961) showed a general agreement except in the case of herbivores which in this experiment showed efficiencies 2-3 times higher than Whittakers and Lindemanns data. Some variation of this sort are to be expected considering the obvious limitations of this laboratory set-up.

The process of aquarium ageing was reflected in the overall pattern of P^{32} movement through the experimental period. The increasing P^{32} content of the mud as P^{32} moved from the liquid to the solid (organism) to the sediment and finally to the mud phase, was one major indication of "ageing". The gradual decline of the sediment weight through the experiment as the millipore biomass decreased was another. Other indications included changes in the type and size of the bottom and sidewall plant communities.

P^{32} UPTAKE AND LOSS EXPERIMENTS

Two experiments involving individual aquatic invertebrate species (Cypris and Limnophilus stigma) were carried out to investigate P^{32} uptake and its subsequent loss in an uncontaminated environment. The most notable features of the Cypris experiment were, the implied concentration ratio of 1.0×10^7 (the highest recorded by any species in all the experiments carried out), the presence of two distinct activity loss rates (r_4) (0.028 uc/gram/hour up to 144 hours and 0.0041 uc/gram/hour from 144 - 480 hours) which was taken to imply the existence of at least two P^{32} micropools with different turnover and loss rates. The results of the Limnophilus stigma experiment exhibited a similar pattern of P^{32} uptake and loss. The rapid initial uptake implied that a large proportion of P^{32} was not assimilated, i.e. that it was externally held to the body surface, probably by bacteria. This observation is supported by the fact that Cypris lost 6% of its P^{32} load after 24 hours in an uncontaminated solution, whereas Limnophilus lost about 50%.

Finally, the experiment has suggested a number of improvements in experimental method. The complexity of the indoor aquaria probably produced an unstable system which was not only difficult to replicate but also very different from a natural community, e.g. it was a closed system containing carnivores which would naturally run down eventually if left undisturbed. A better system for laboratory analysis would have to be simpler and easier to replicate. Such a system was set up but it took nearly six months before any self regenerating stability appeared. This system held some 50 litres of pond water along with two species of mollusc (Limnea and Planorbis), Daphnia, Cyclops, Cypris, Diaptomus, Chironomid and Oligochaete populations in the mud, phytoplankton, a sidewall community, Duckweed (Lemna trisulca) and an amount of Elodea. This system progressed through to eventual stagnation, however within two months a stable community appeared which would have been suitable for analysis. Naturally, sampling weights would have to be fairly small, however this should be offset by using higher P^{32} tag levels.

Isolated field populations within large plastic framed containers set about one foot into the mud would be ideal for tracer study in the wild over prolonged periods of several months. Replicates could be easily set up and the isotope isolated from the main area of the water. Drawbacks include the lack of movement of organisms through the tank, the possible overexploitation of one species by another, e.g. Sticklebacks, and the effect of the algal populations which would cover the plastic sidewalls. To varying degrees, these problems could be overcome. Also the P^{32} concentration could be kept around a constant level by some sort of drip feed or spray device every 24 hours or so in order to compensate for P^{32} lost by the half life decay.

Finally, a more effective method of separation is needed in certain fractions, e.g. mud, plankton, sidewalls, along with detailed analyses of P^{32} "states", i.e. chemical forms.

A C K N O W L E D G M E N T S

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Errata:

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FIGURES

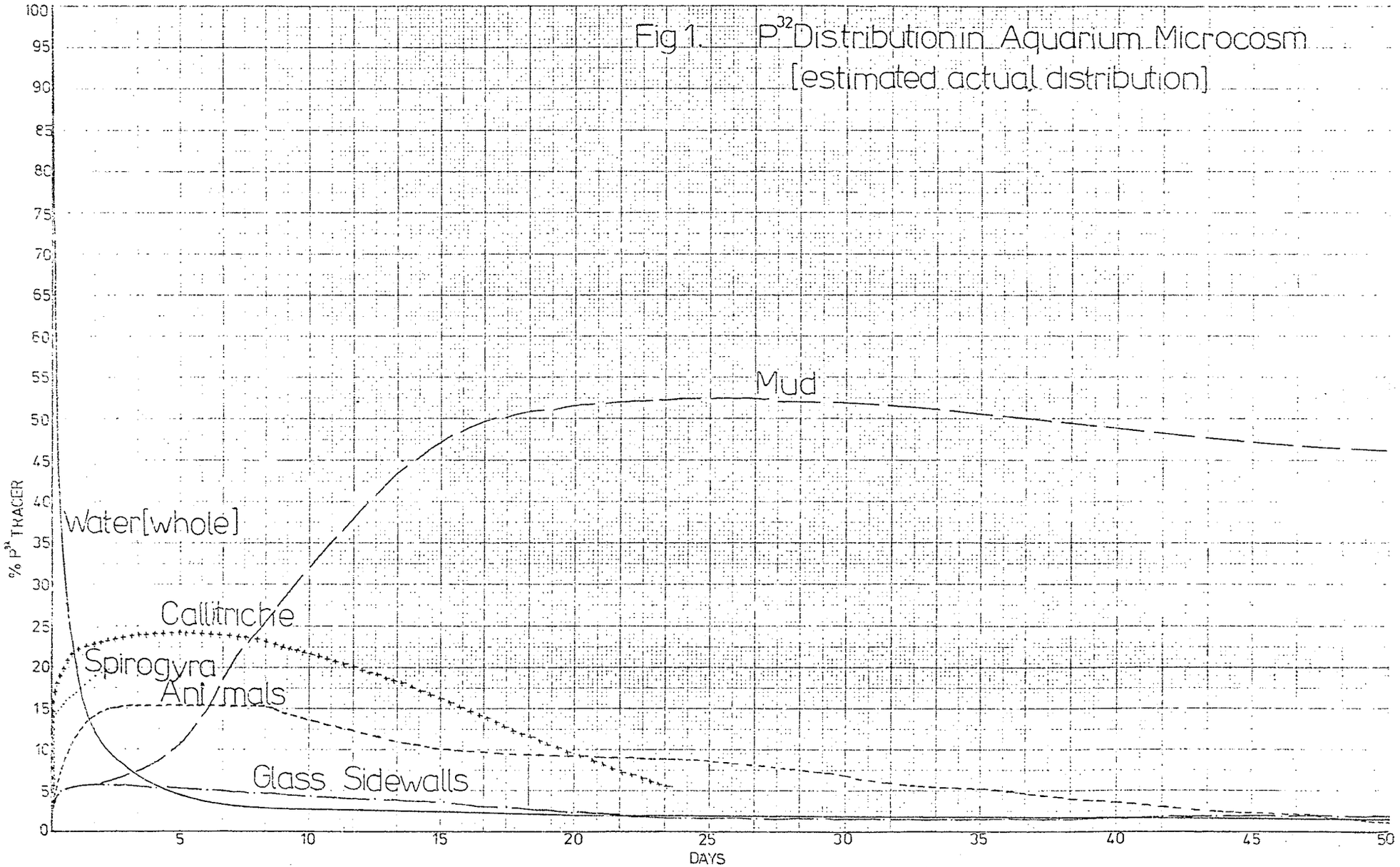


Fig 1. P32 Distribution in Aquarium Microcosm
[estimated actual distribution]
% P32 TRACER
DAYS
Mud
Water[whole]
Callitriche
Spirogyra
Animals
Glass Sidewalls
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100

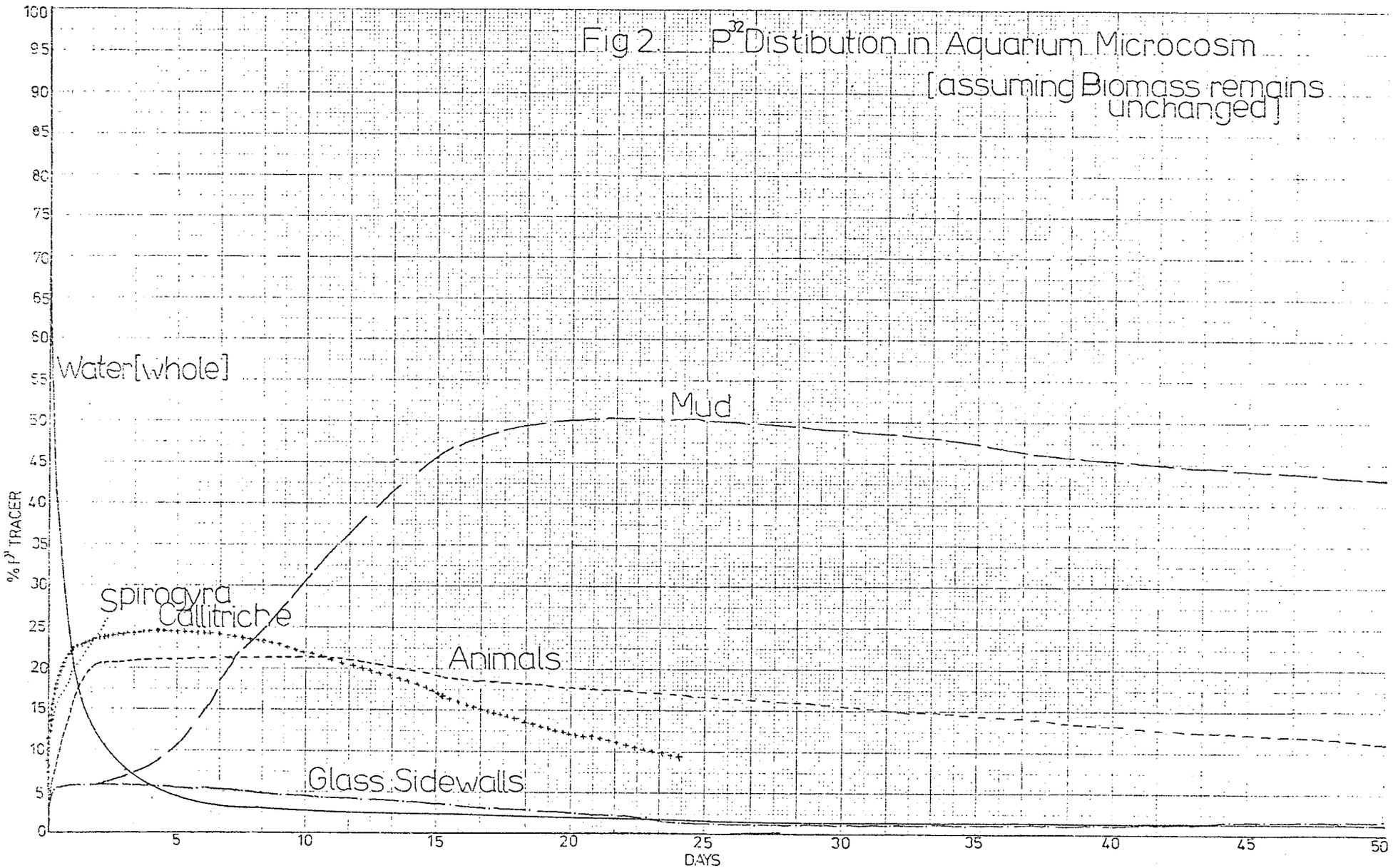


Fig 2 P32 Distibution in Aquarium Microcosm
[assuming Biomass remains unchanged]
Water[whole]
Mud
Spirogyra
Callitriche
Animals
Glass Sidewalls
% P32 TRACER
DAYS
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100

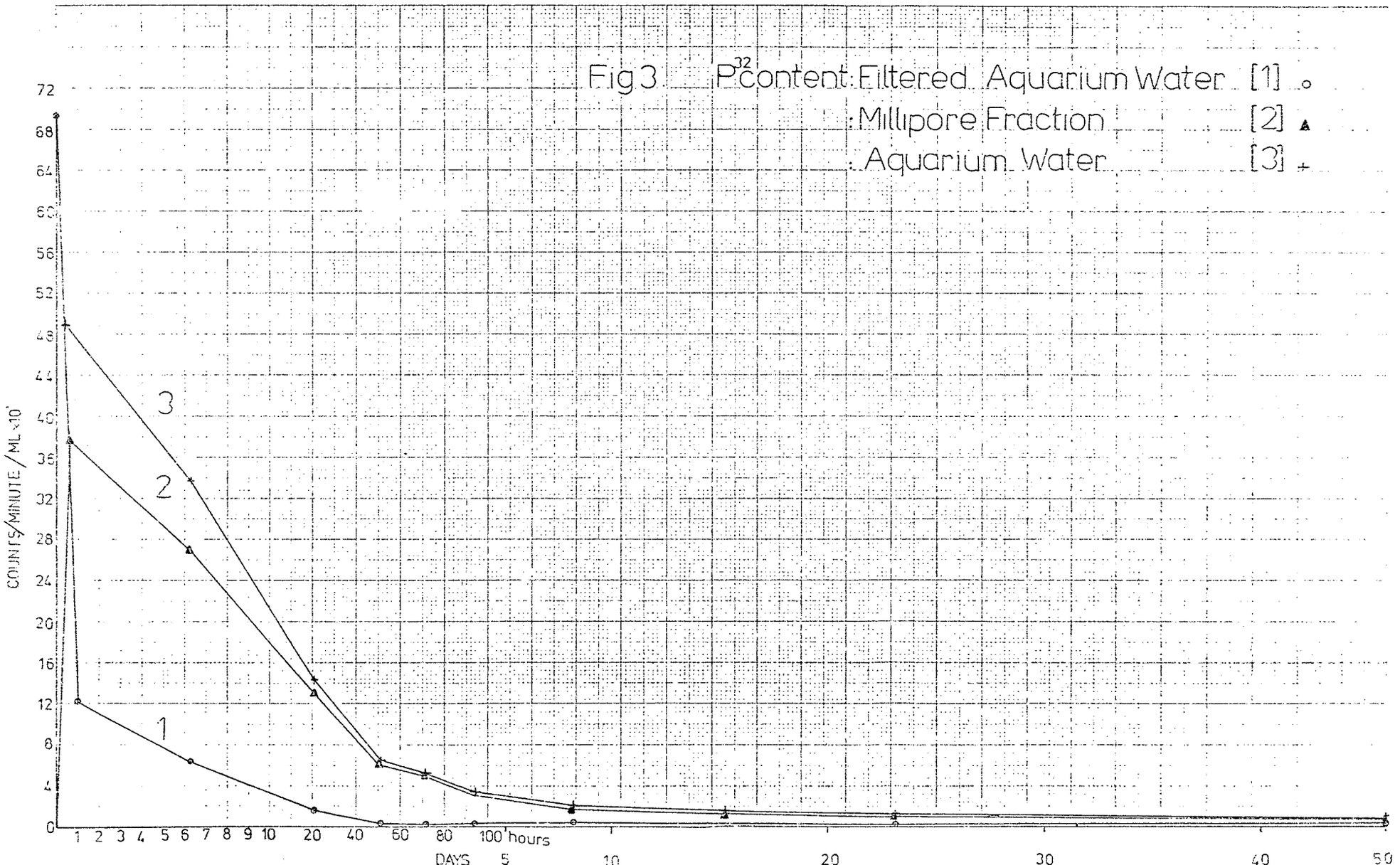


Fig 3 P^{32} content: Filtered Aquarium Water [1] ∘
Millipore Fraction [2] ▲
Aquarium Water [3] +
COUNTS/MINUTE/ML x10
72
68
64
60
56
52
48
44
40
36
32
28
24
20
16
12
8
4
0
1
2
3
1 2 3 4 5 6 7 8 9 10 20 40 60 80 100 hours
DAYS 5 10 20 30 40 50

Fig 4. P^{32} uptake in Aquarium Microcosm

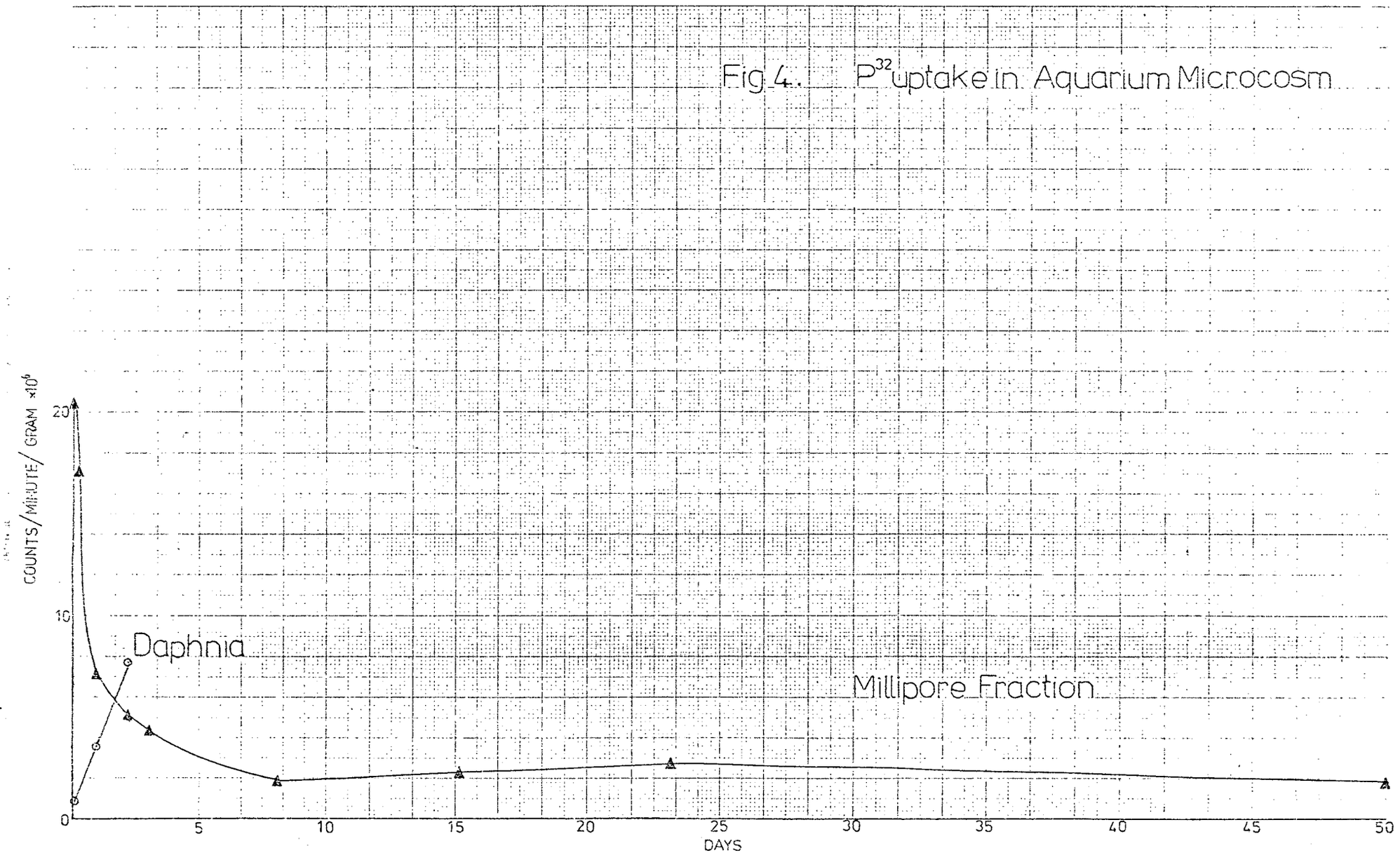
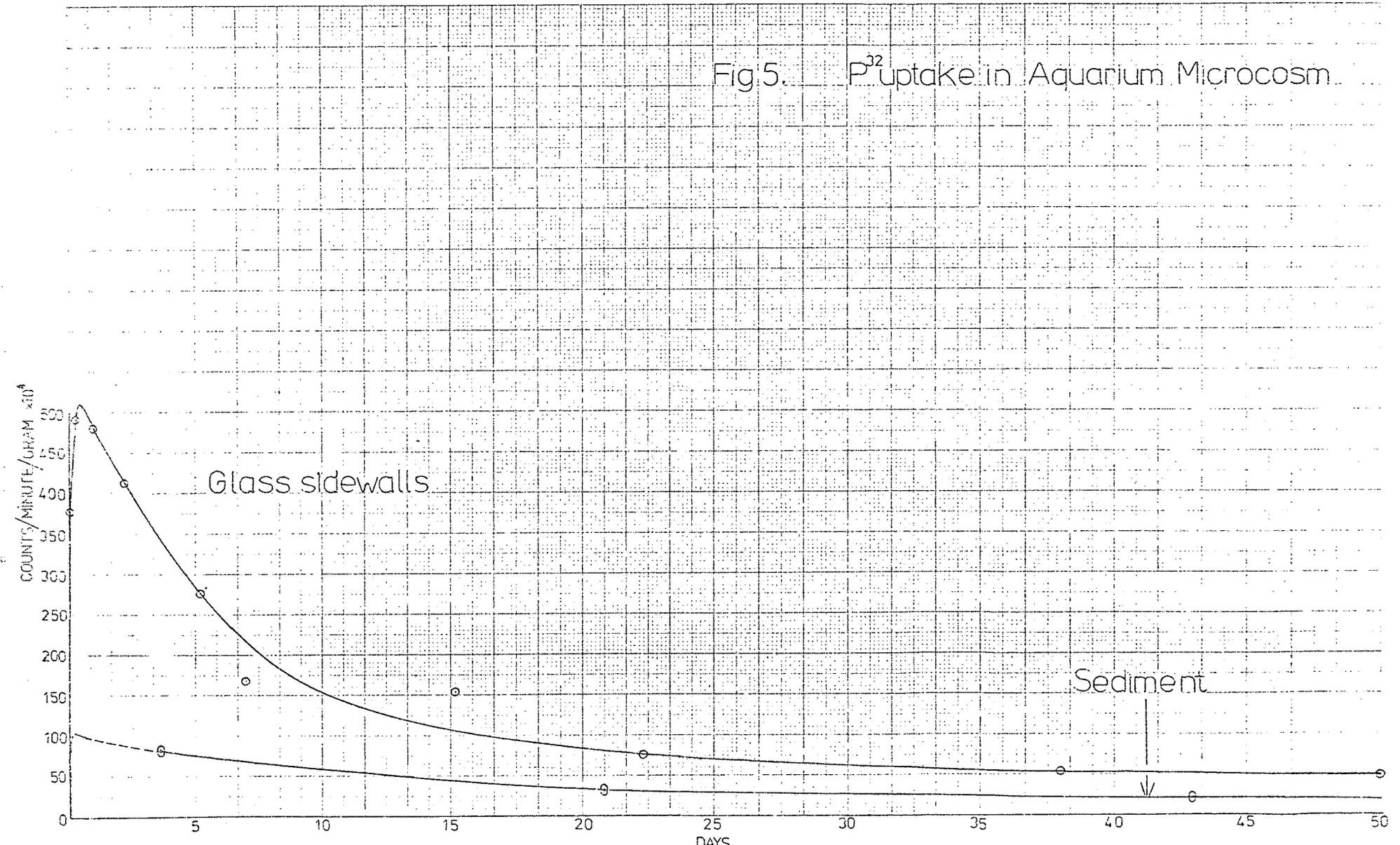


Fig 5. P^{32} uptake in Aquarium Microcosm



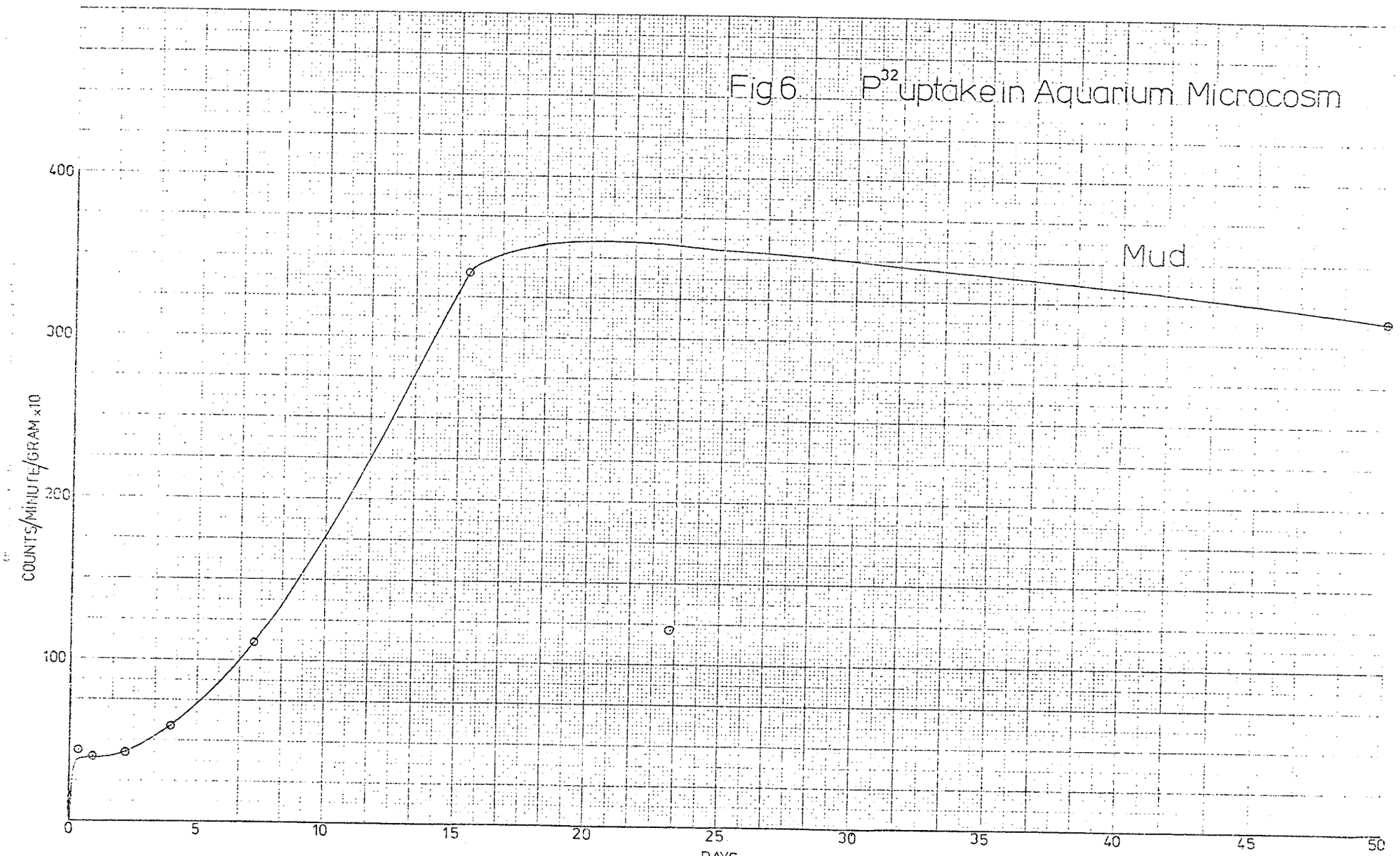


Fig 6 P^{32} uptake in Aquarium Microcosm
Mud
COUNTS/MINUTE/GRAM x10
400
300
200
100
0
5
10
15
20
25
30
35
40
45
50
DAYS

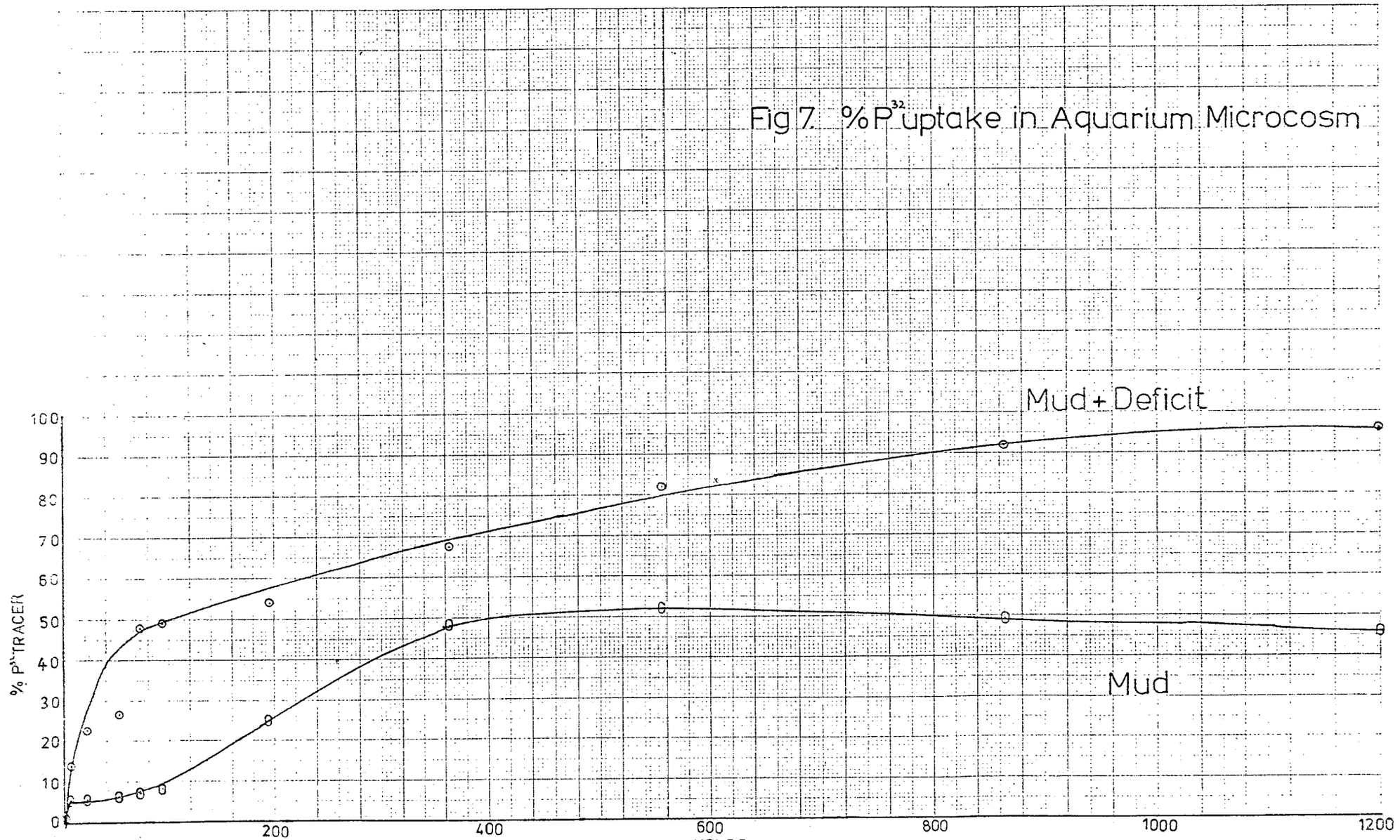
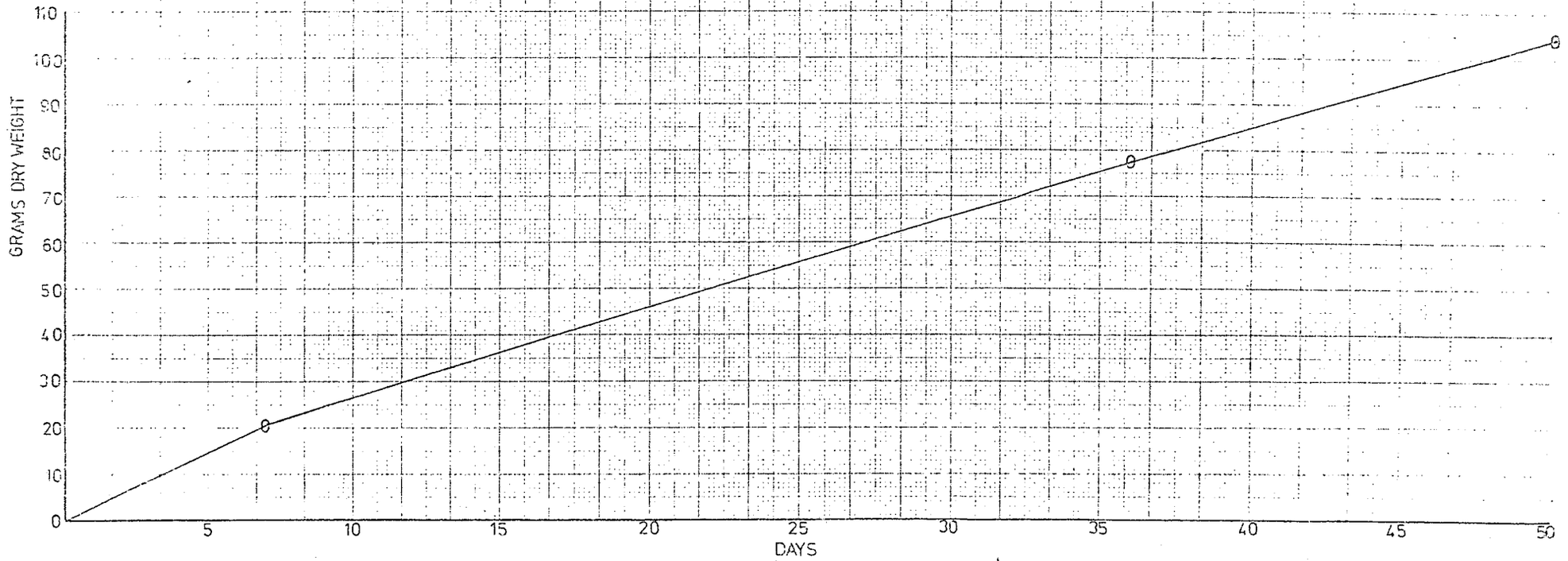


Fig 7 % P32 uptake in Aquarium Microcosm
Mud + Deficit
Mud
% P32 TRACER
0
10
20
30
40
50
60
70
80
90
100
200
400
600
800
1000
1200

Fig 8. Sediment accumulation in Aquarium Microcosm



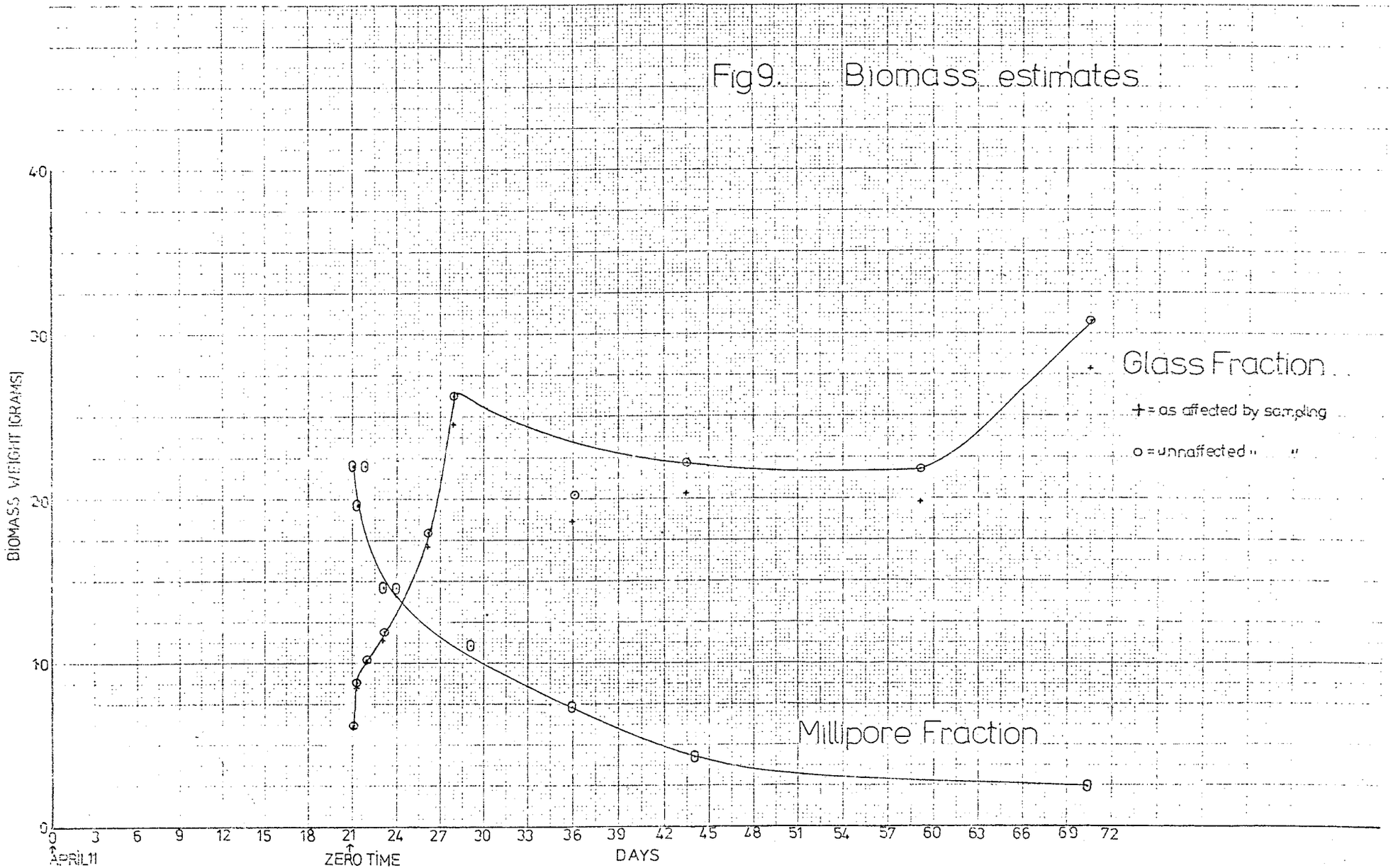


Fig 9. Biomass estimates
BIOMASS WEIGHT (GRAMS)
40
30
20
10
0
Glass Fraction
+ = as affected by sampling
o = unnaffected " "
Millipore Fraction
0 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60 63 66 69 72
APRIL 11
ZERO TIME
DAYS

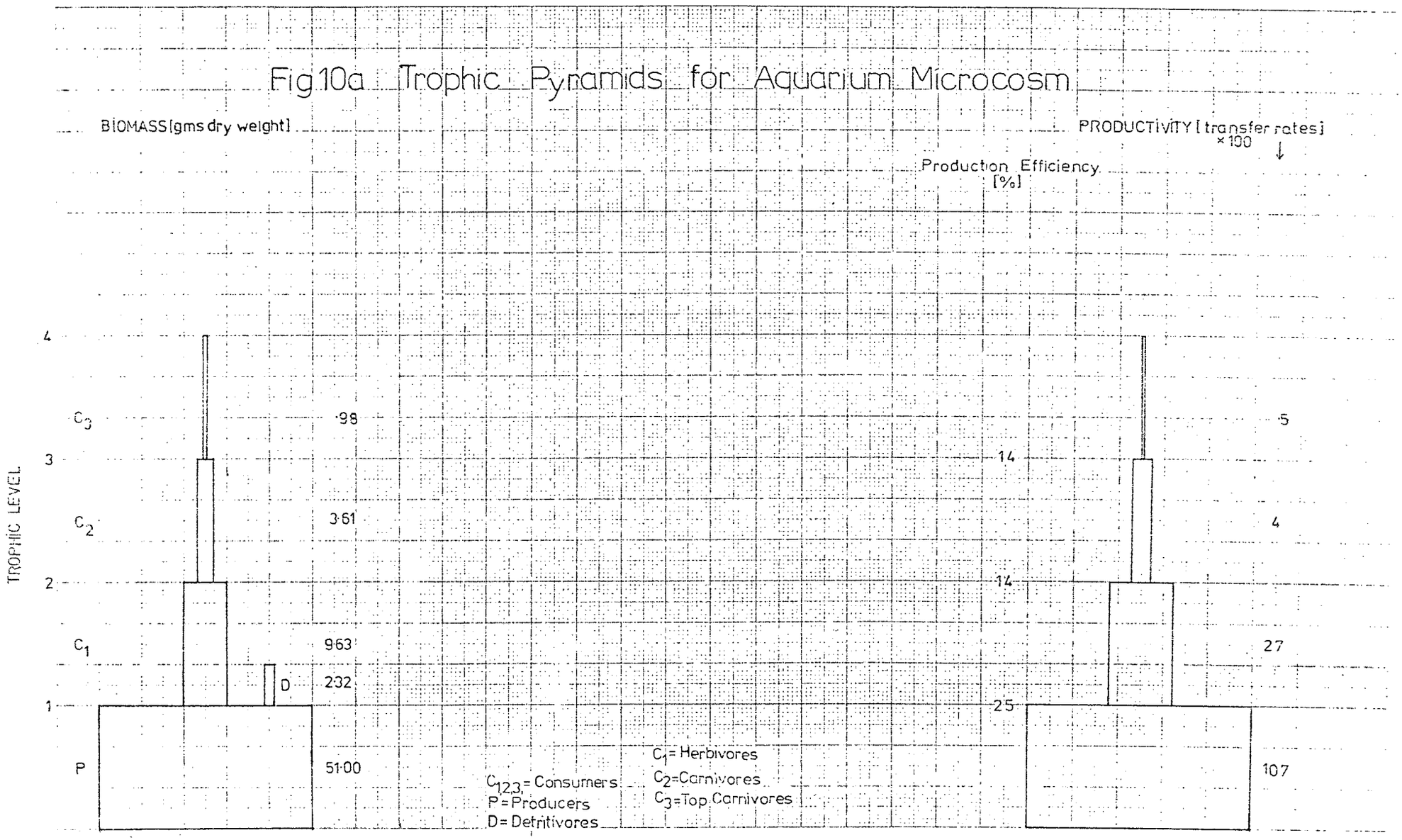


Fig 10a Trophic Pyramids for Aquarium Microcosm
BIOMASS [gms dry weight]
PRODUCTIVITY [transfer rates] ×100
Production Efficiency [%]
TROPHIC LEVEL
4
3
2
1
C3
C2
C1
D
P
·98
3·61
9·63
2·32
51·00
14
14
25
5
4
27
107
C1 = Herbivores
C2 = Carnivores
C3 = Top Carnivores
C1,2,3 = Consumers
P = Producers
D = Detritivores

Fig10b MAJOR P^{32} CYCLING PATHWAYS IN AQUARIUM MICROCOSM EXPERIMENT AND TRANSFER RATES

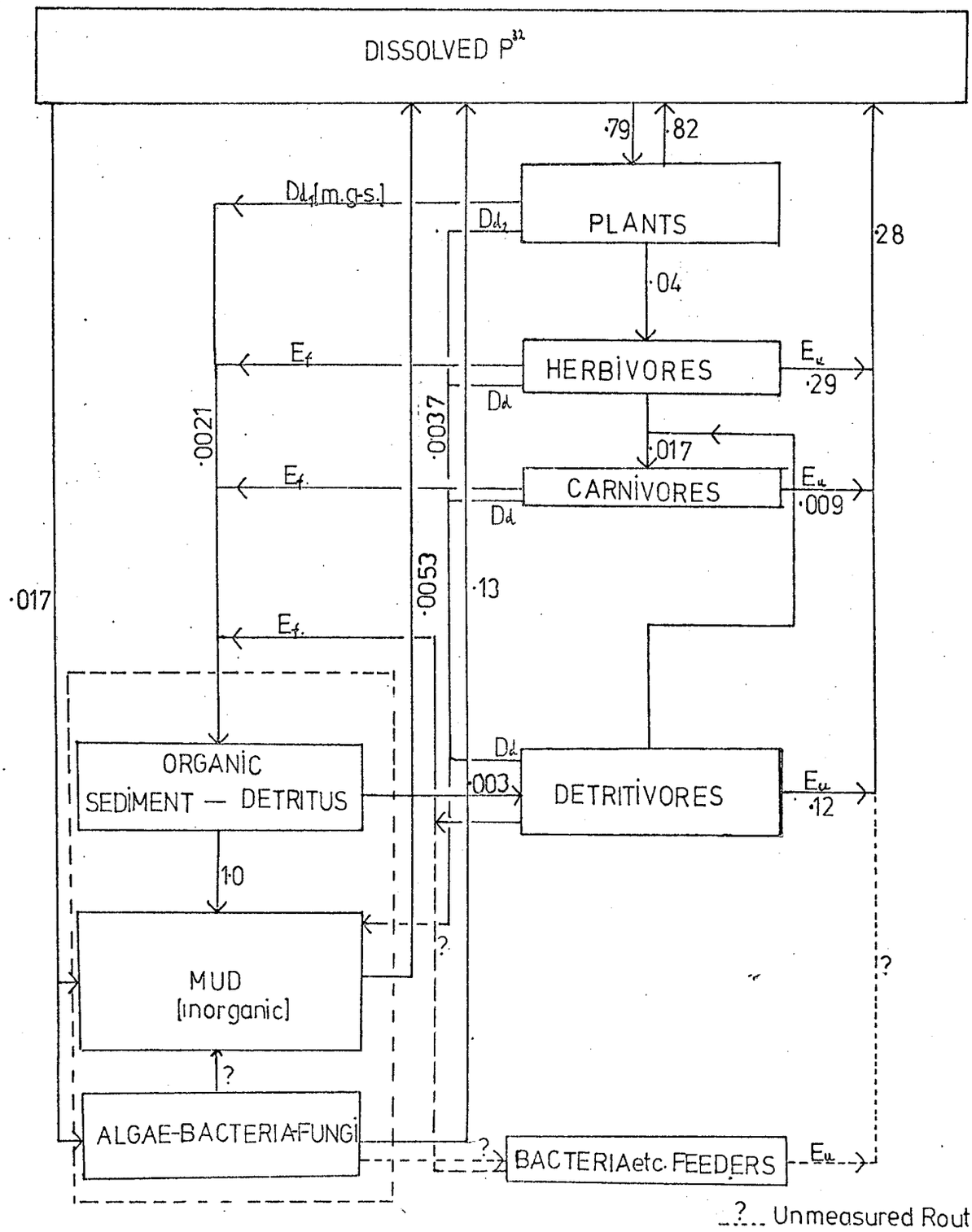


Fig 10c P^{32} CYCLING IN THE AQUARIUM MICROCOSM

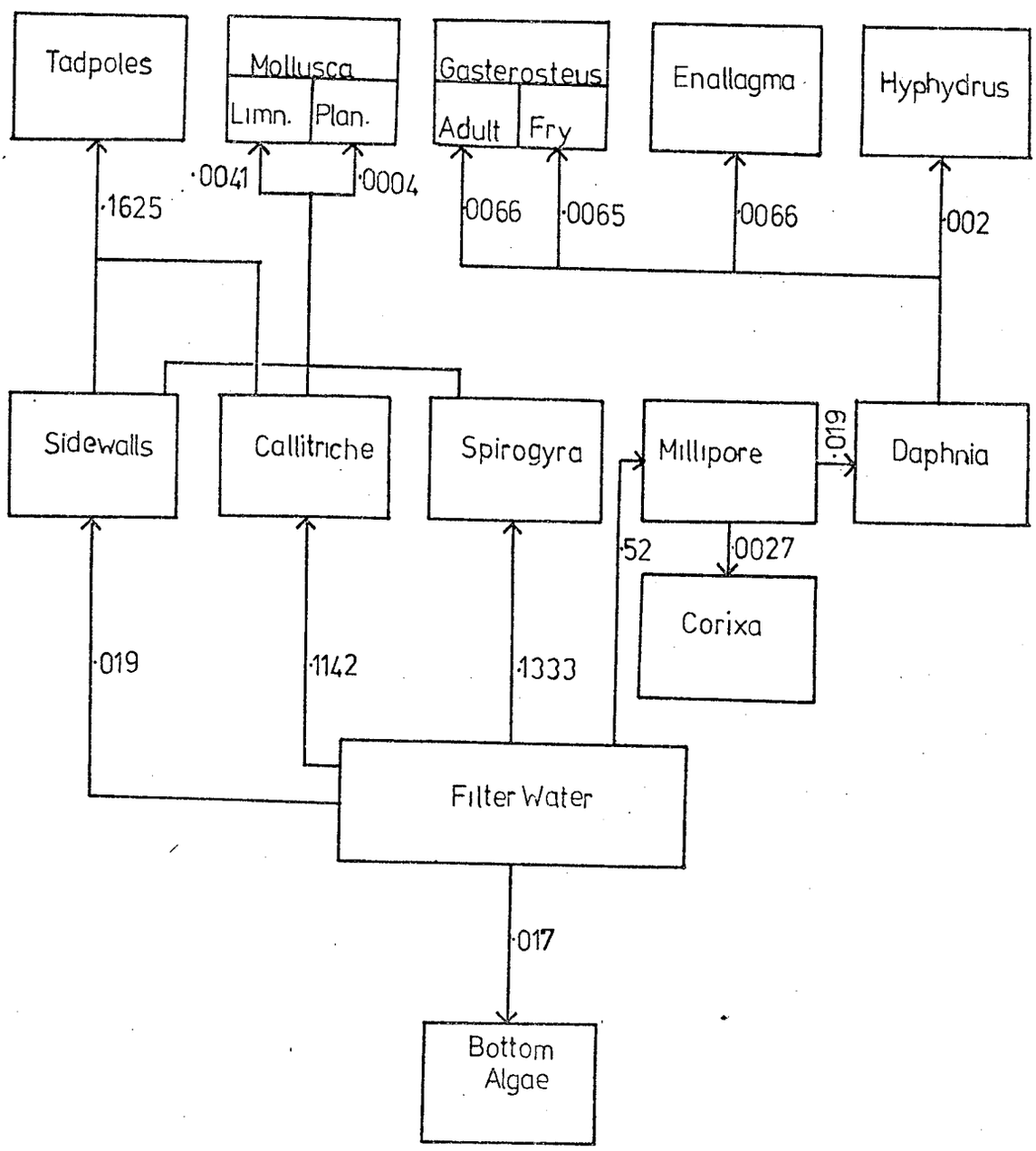
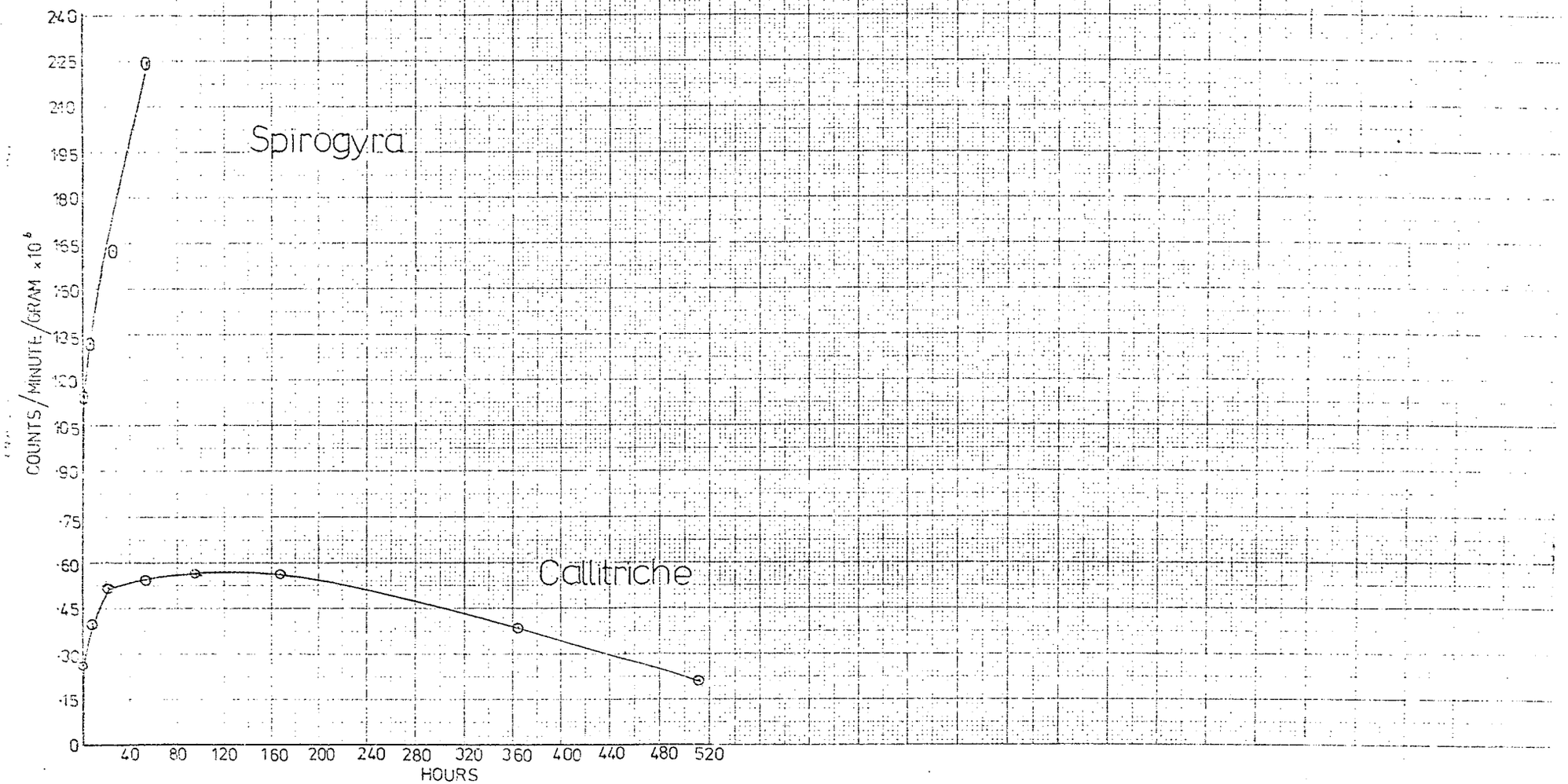


Fig 11. P^{32} uptake in Aquarium Microcosm



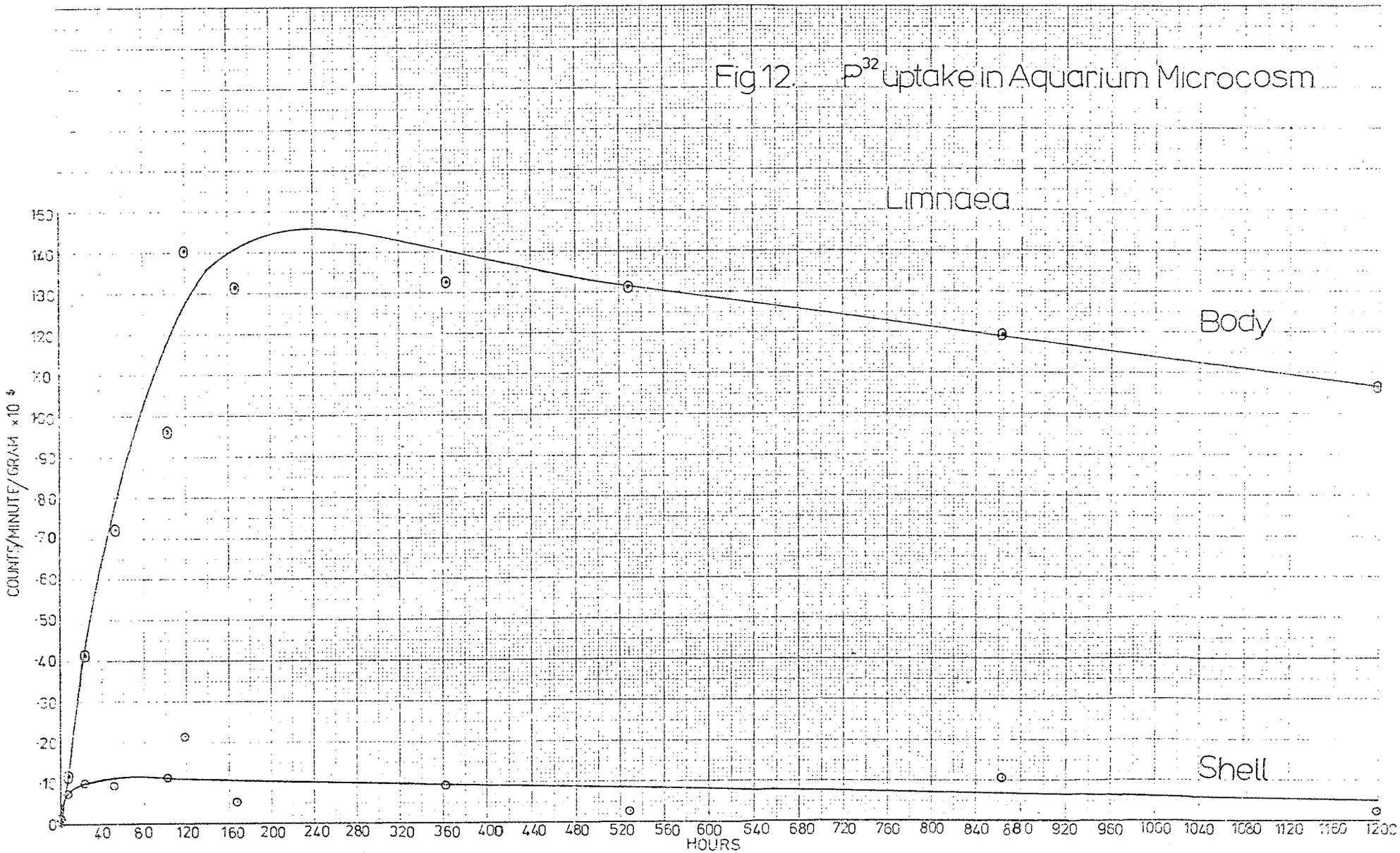
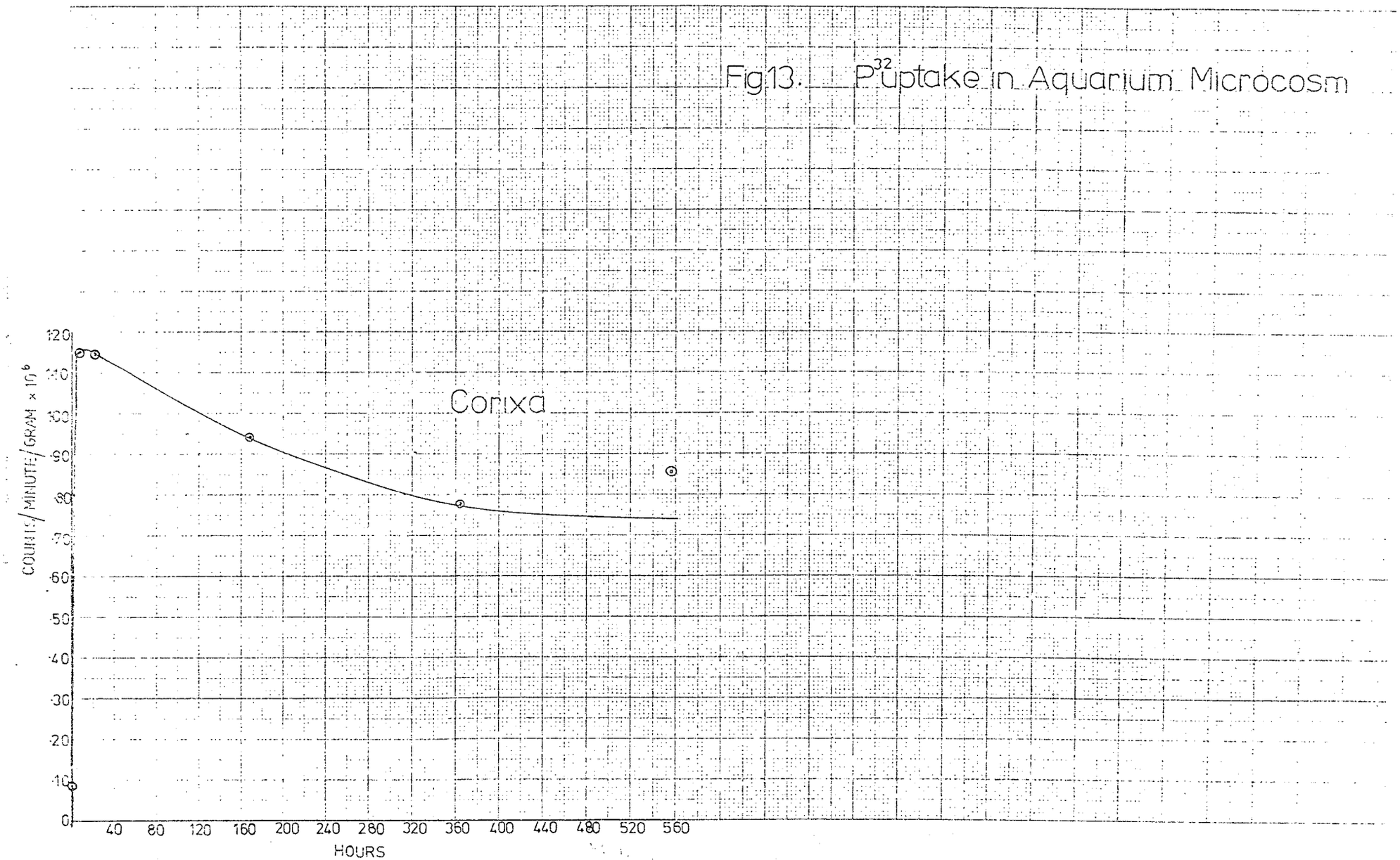


Fig.12 P^{32} uptake in Aquarium Microcosm
Limnaea
Body
Shell
COUNTS/MINUTE/GRAM ×10 ⁴
HOURS
0
40
80
120
160
200
240
280
320
360
400
440
480
520
560
600
640
680
720
760
800
840
880
920
960
1000
1040
1080
1120
1160
1200

Fig 13. P^{32} uptake in Aquarium Microcosm



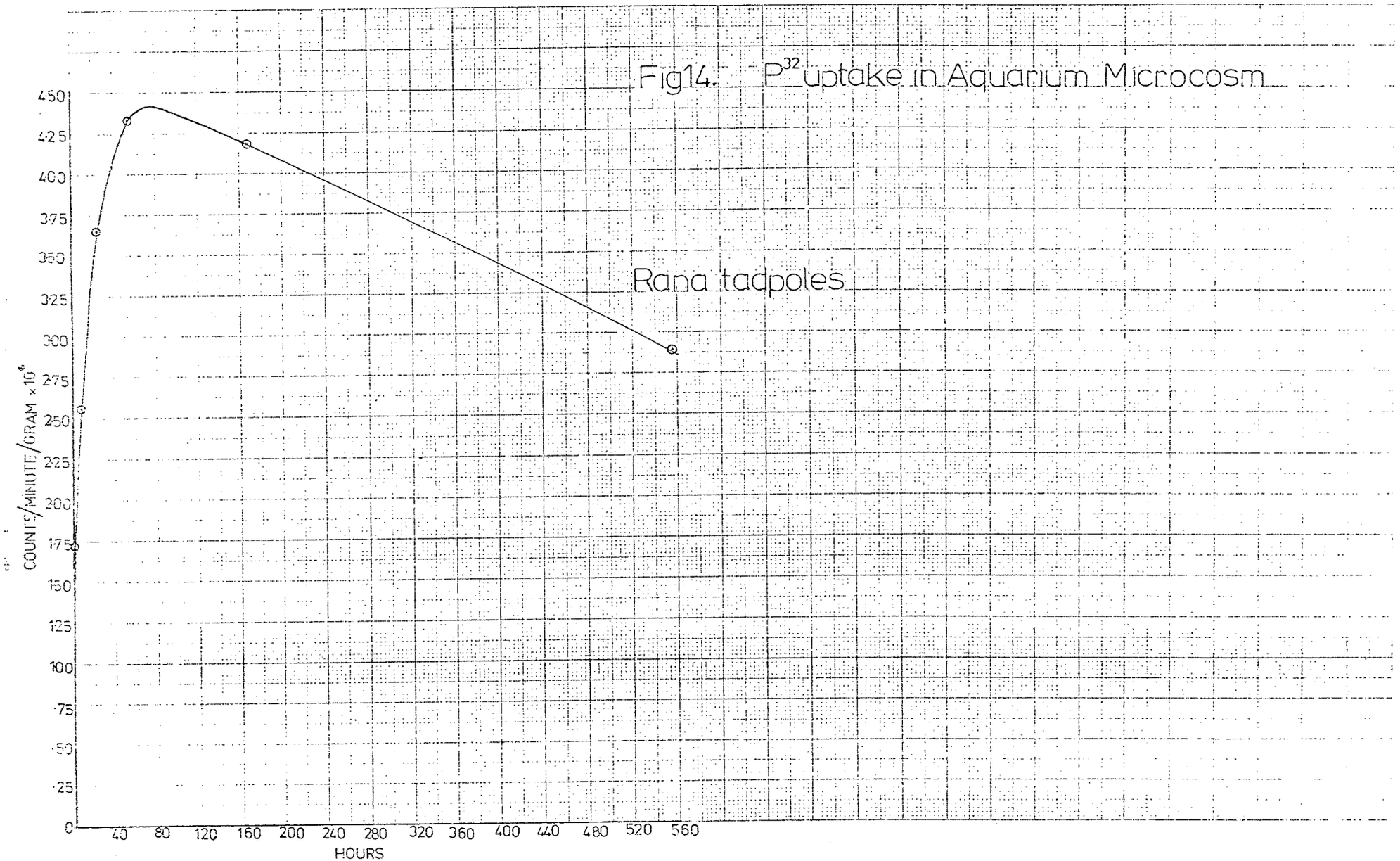


Fig14. P^{32} uptake in Aquarium Microcosm
Rana tadpoles
COUNTS/MINUTE/GRAM $\times 10^6$
450
425
400
375
350
325
300
275
250
225
200
175
150
125
100
75
50
25
0
40
80
120
160
200
240
280
320
360
400
440
480
520
560
HOURS

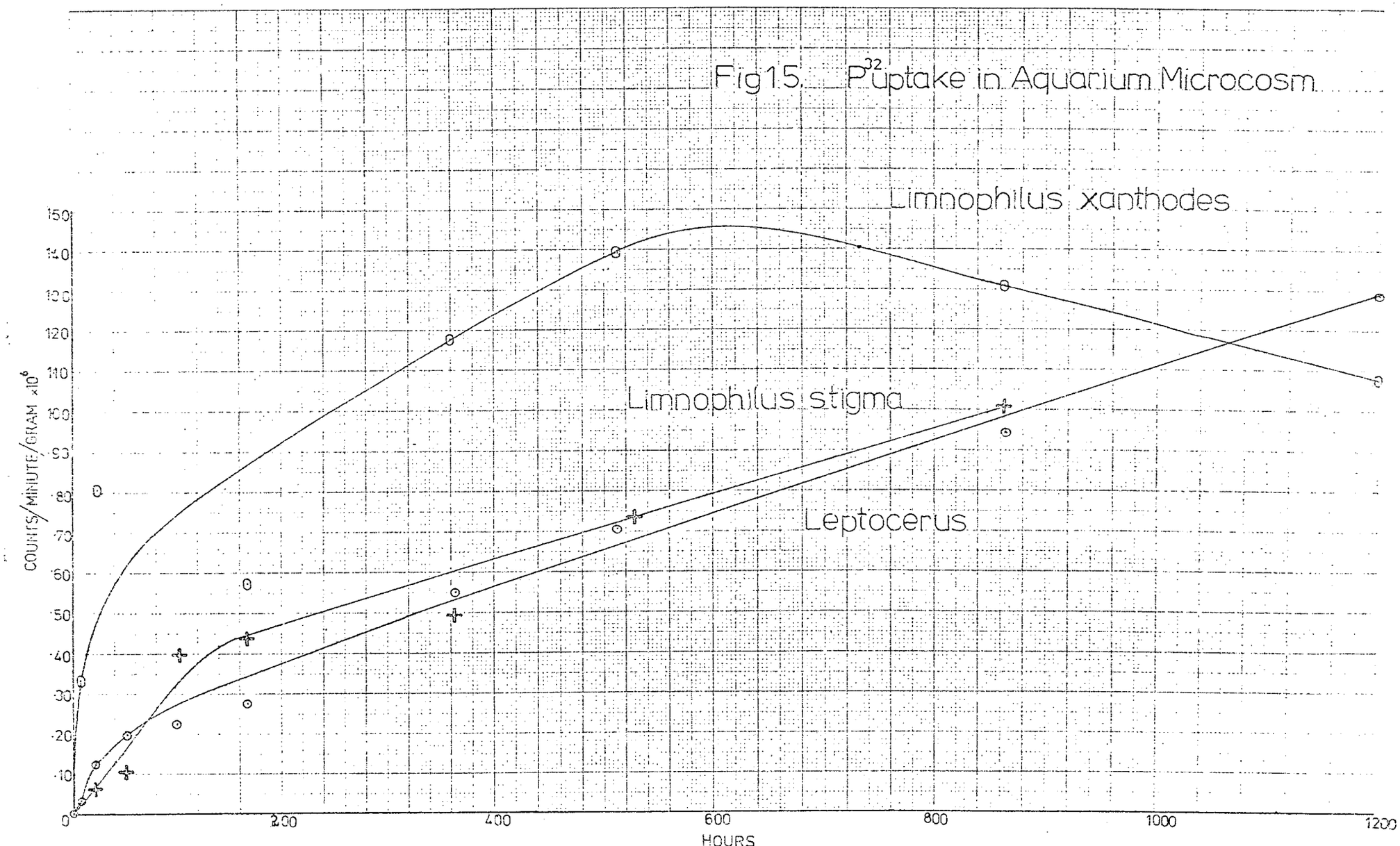


Fig 15. P^{32} uptake in Aquarium Microcosm
Limnophilus xanthodes
Limnophilus stigma
Leptocerus
COUNTS/MINUTE/GRAM ×10⁶
HOURS
0
200
400
600
800
1000
1200

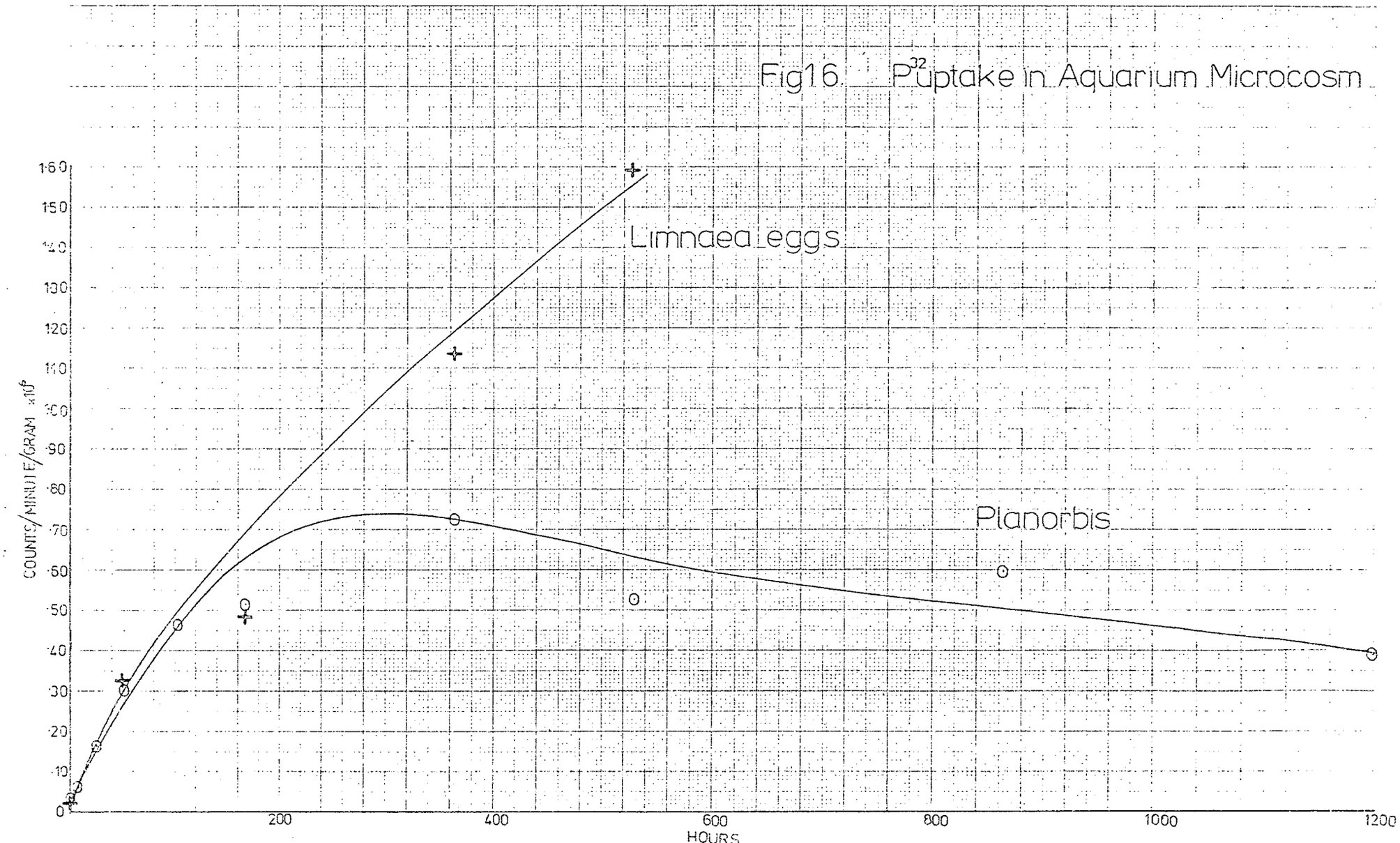


Fig16 P^{32} uptake in Aquarium Microcosm
Limnaea eggs
Planorbis
COUNTS/MINUTE/GRAM x10³
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
200
400
600
800
1000
1200
HOURS

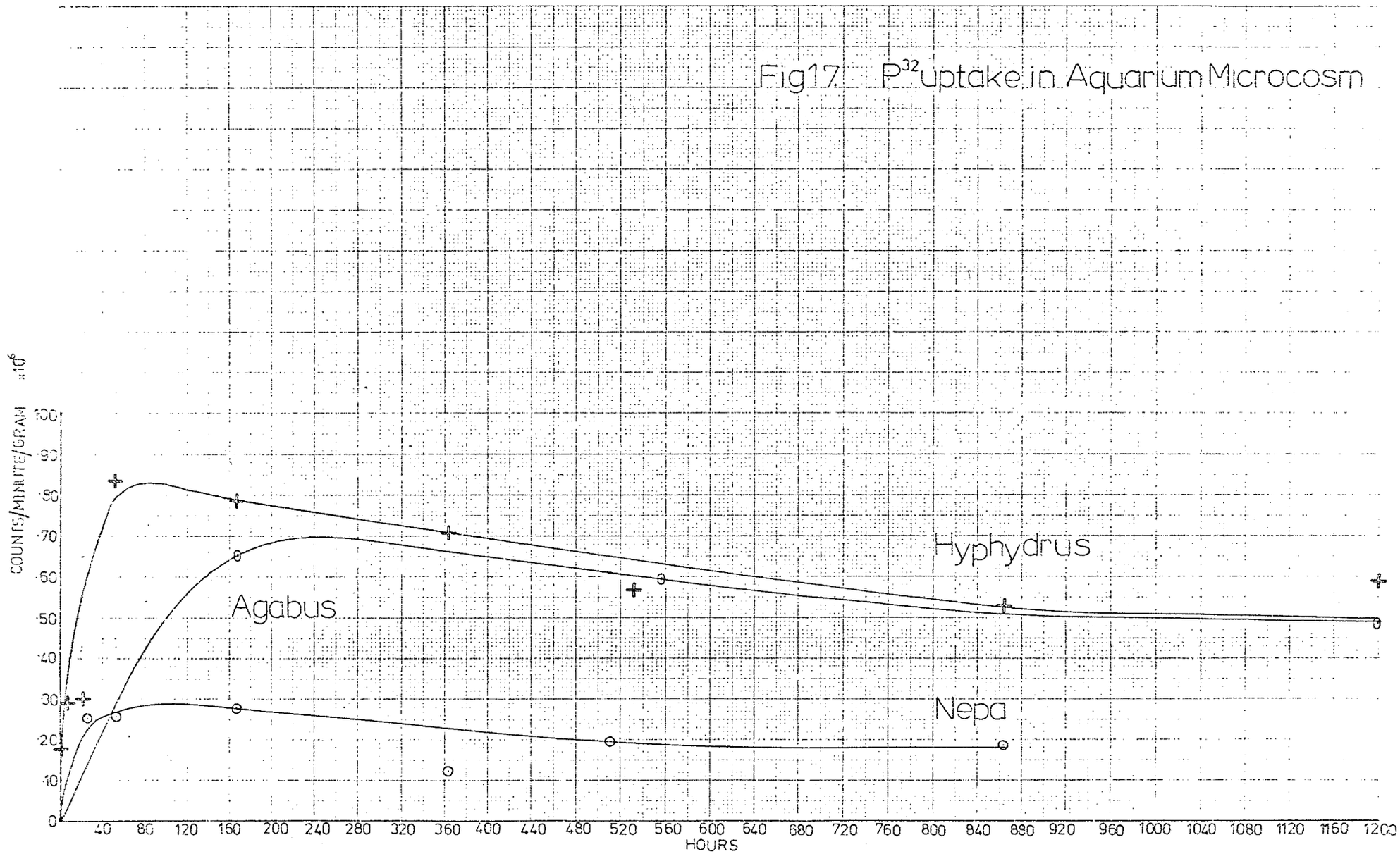


Fig17 P32 uptake in Aquarium Microcosm
COUNTS/MINUTE/GRAM x10^6
100
90
80
70
60
50
40
30
20
10
0
Hyphydrus
Agabus
Nepa
40 80 120 160 200 240 280 320 360 400 440 480 520 560 600 640 680 720 760 800 840 880 920 960 1000 1040 1080 1120 1160 1200
HOURS

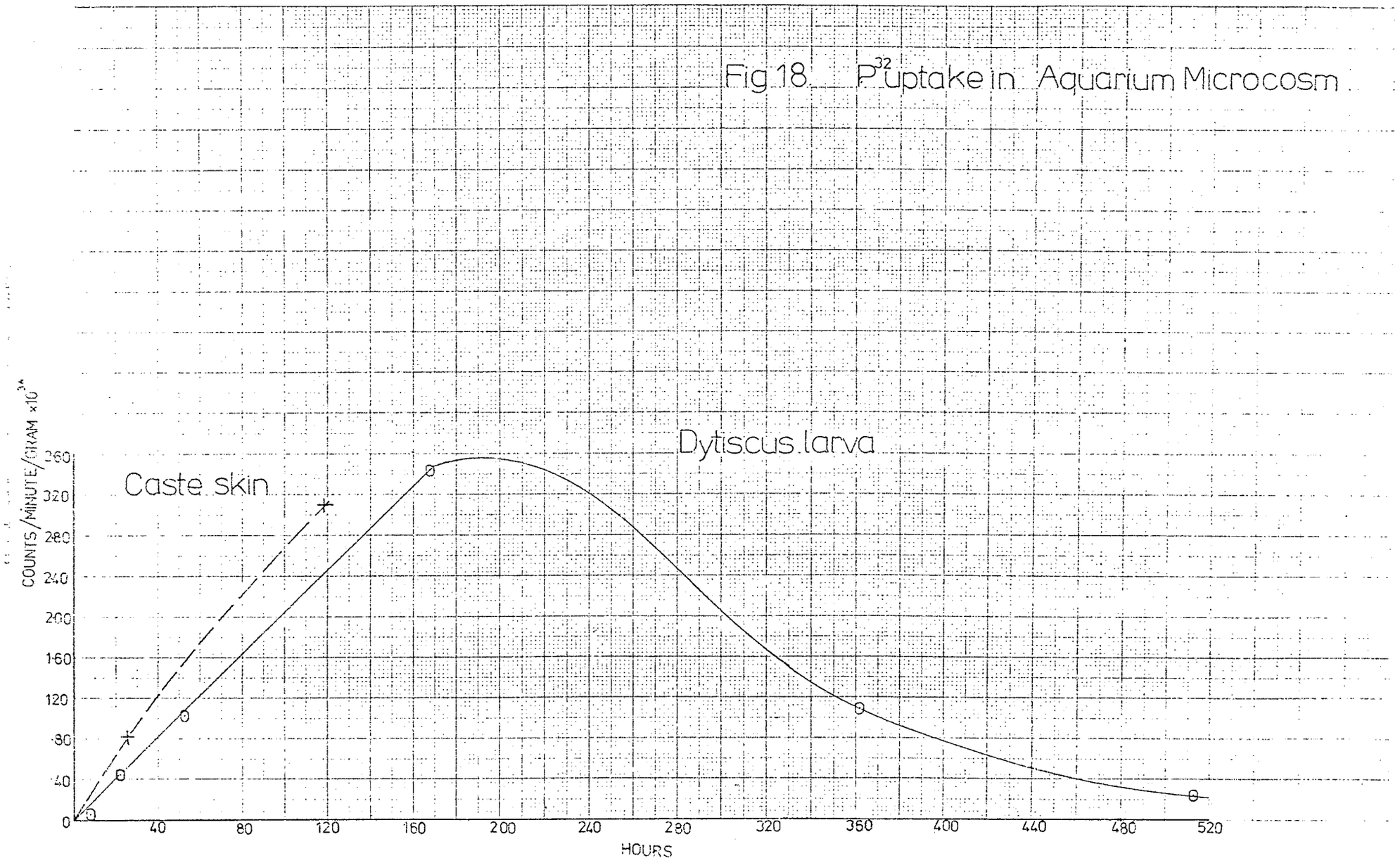
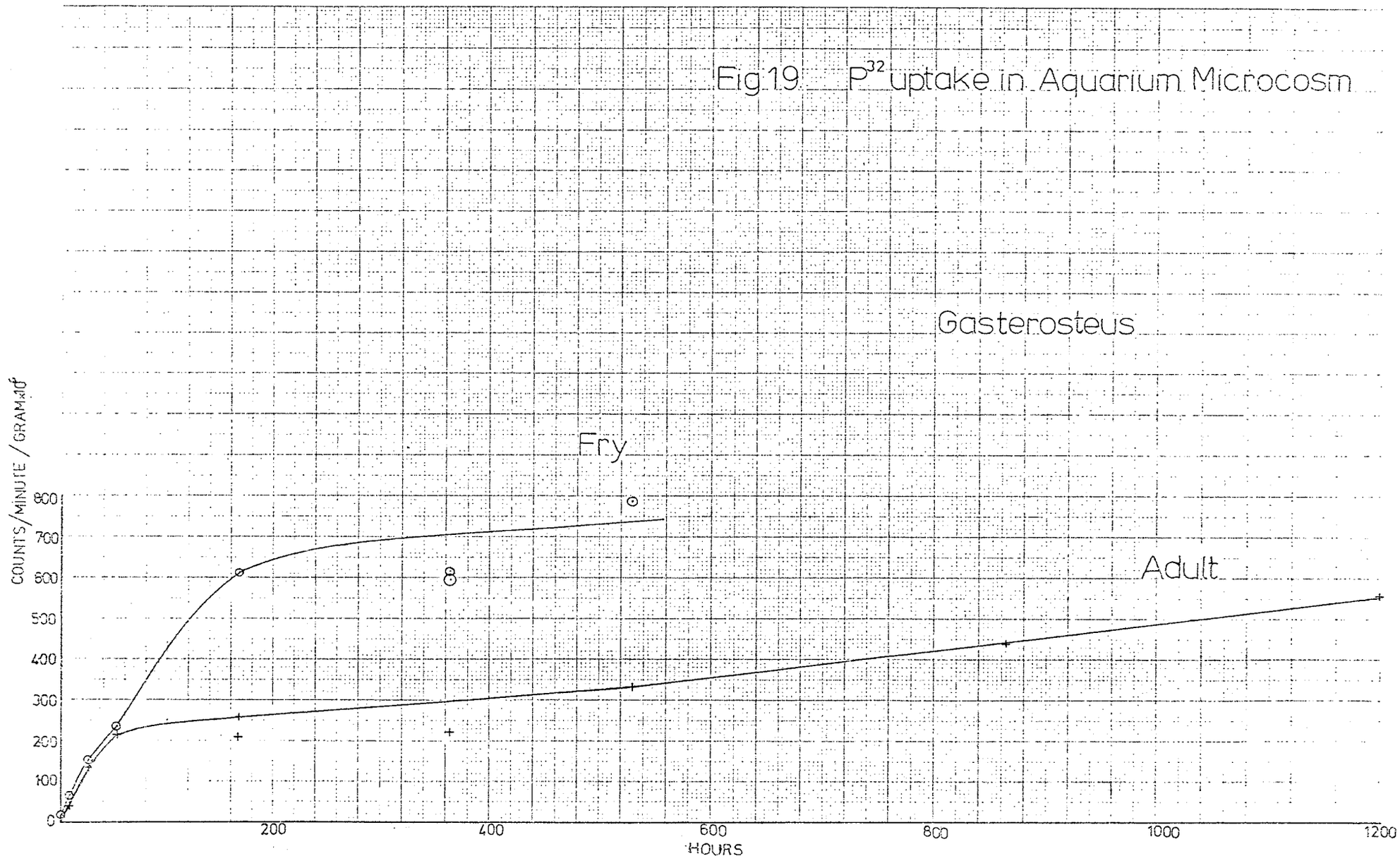


Fig 18 P32 uptake in Aquarium Microcosm
COUNTS/MINUTE/GRAM x10^3
360
320
280
240
200
160
120
80
40
0
Caste skin
Dytiscus larva
40
80
120
160
200
240
280
320
360
400
440
480
520
HOURS

Fig 19 P^{32} uptake in Aquarium Microcosm



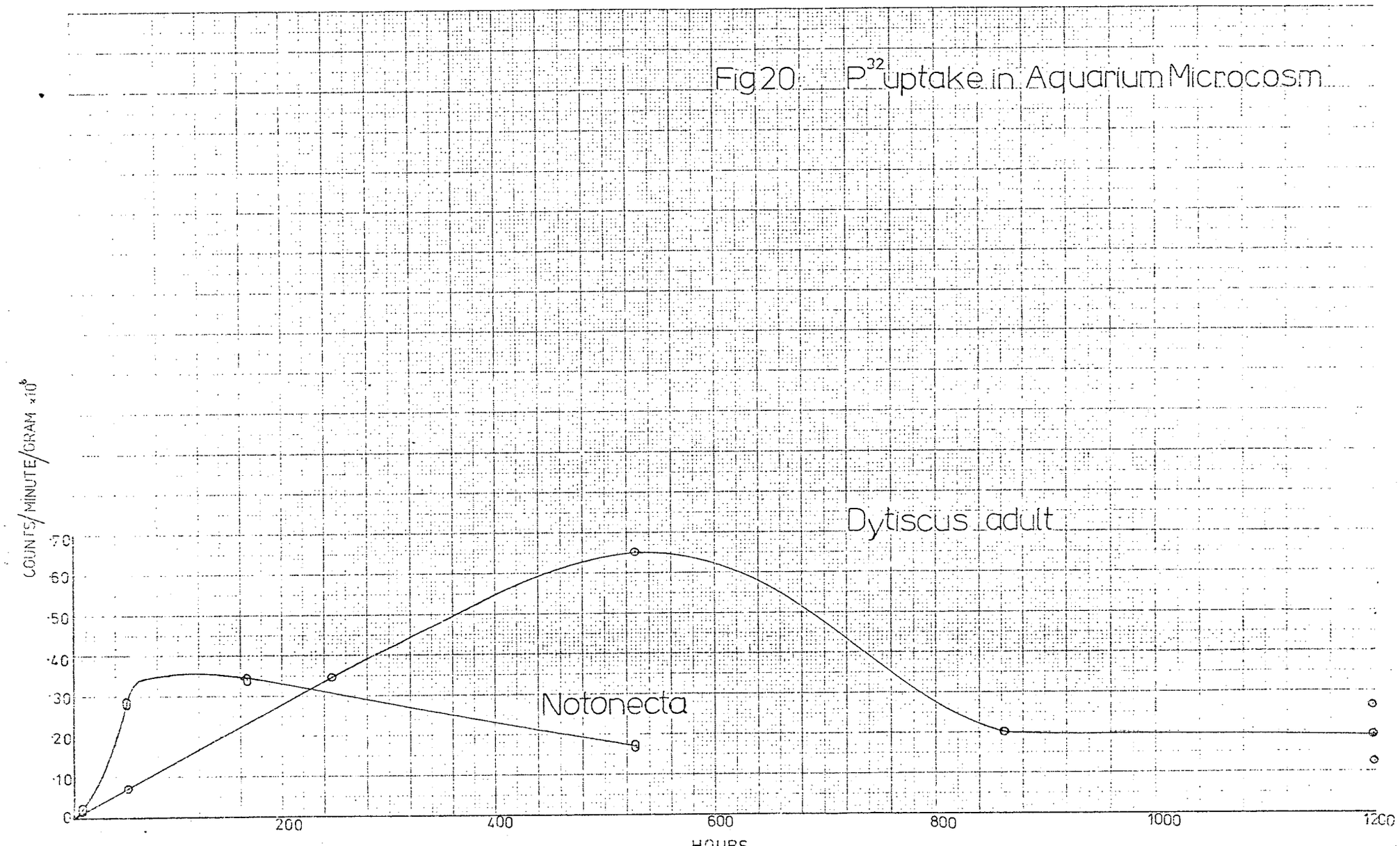


Fig 20 P32 uptake in Aquarium Microcosm
COUNTS/MINUTE/GRAM ×10⁶
70
60
50
40
30
20
10
0
200
400
600
800
1000
1200
HOURS
Dytiscus adult
Notonecta

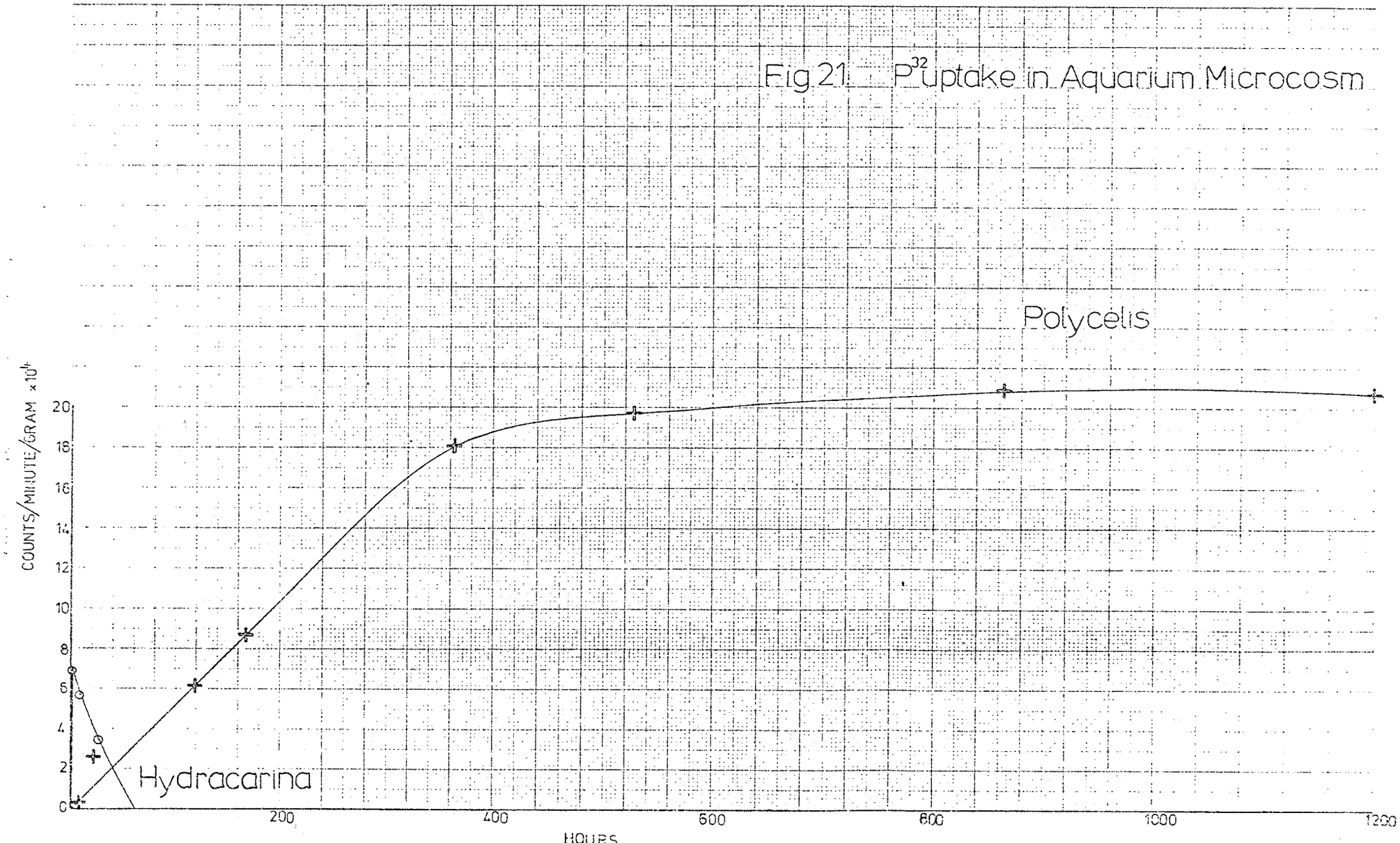


Fig.21 P^{32} uptake in Aquarium Microcosm
Polycelis
Hydracarina
COUNTS/MINUTE/GRAM ×10⁴
0
2
4
6
8
10
12
14
16
18
20
200
400
600
800
1000
1200
HOURS

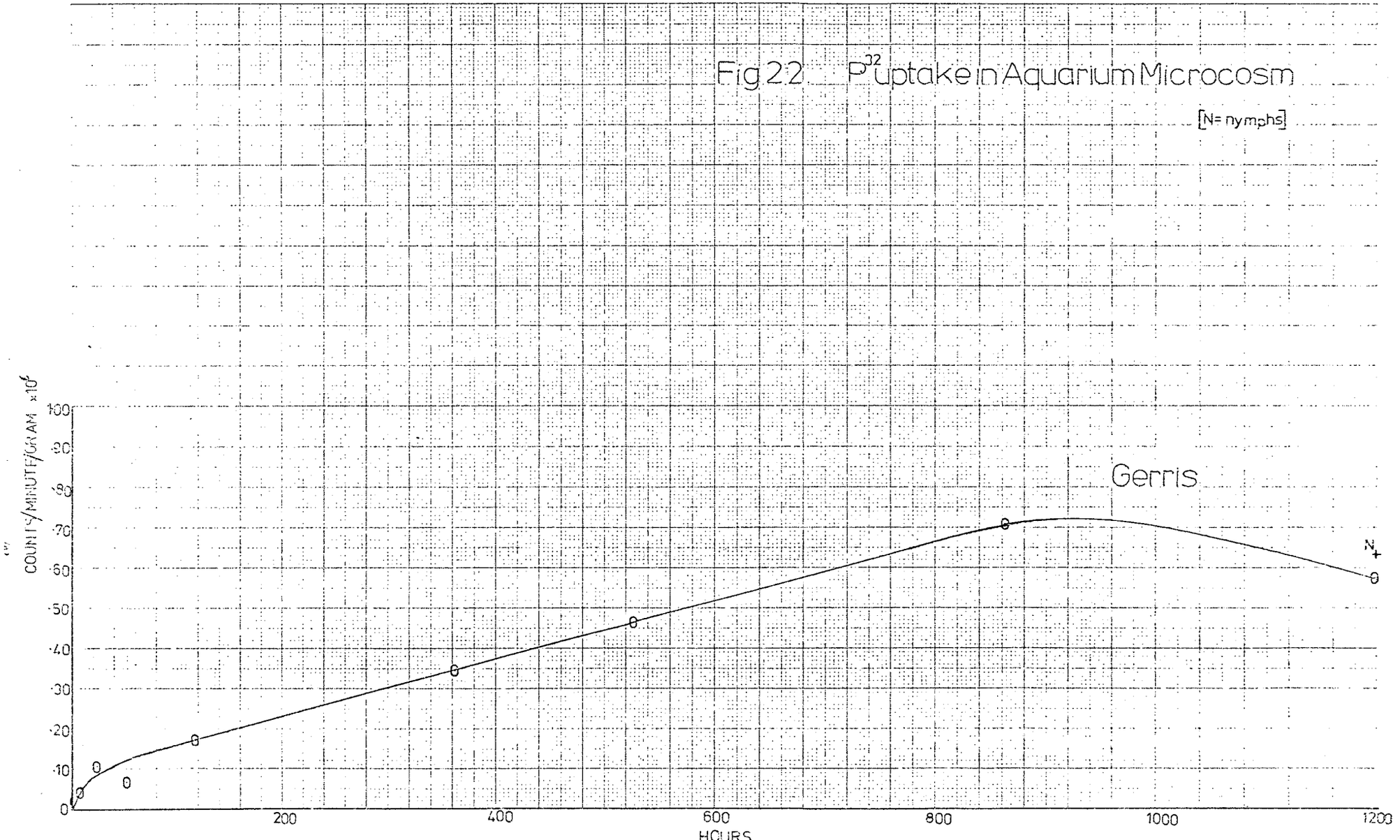


Fig 22 P32 uptake in Aquarium Microcosm
[N= nymphs]
Gerris
COUNTS/MINUTE/GRAM x10⁶
100
90
80
70
60
50
40
30
20
10
0
200
400
600
800
1000
1200
HOURS
N+

Fig 23 P^{32} uptake in Aquarium Microcosm

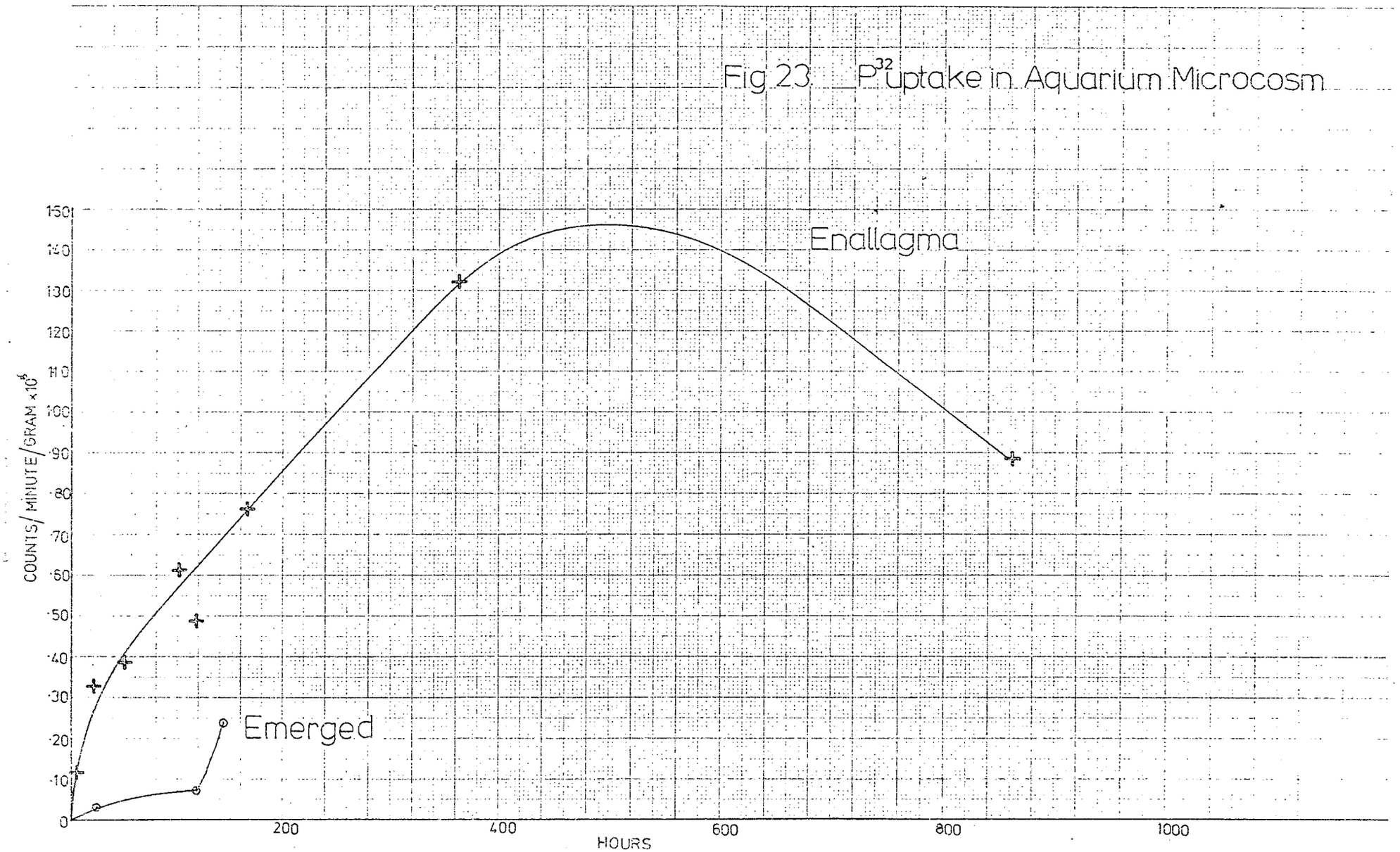


Fig 24 P^{32} uptake in Aquarium Microcosm

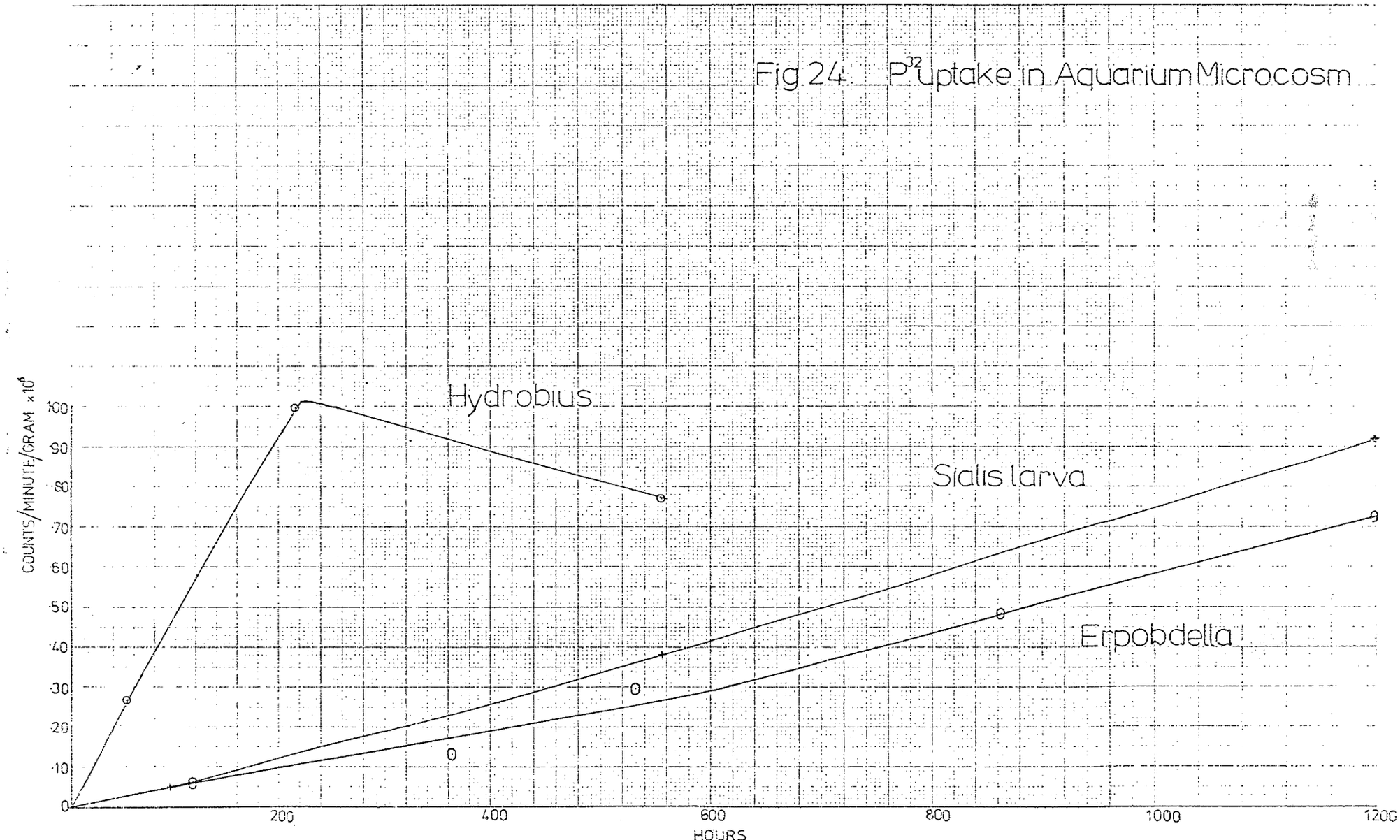


Fig 25. P^{32} content : Biological variation in Hyphydrus ovatus individuals at 50 days [1200 hour]

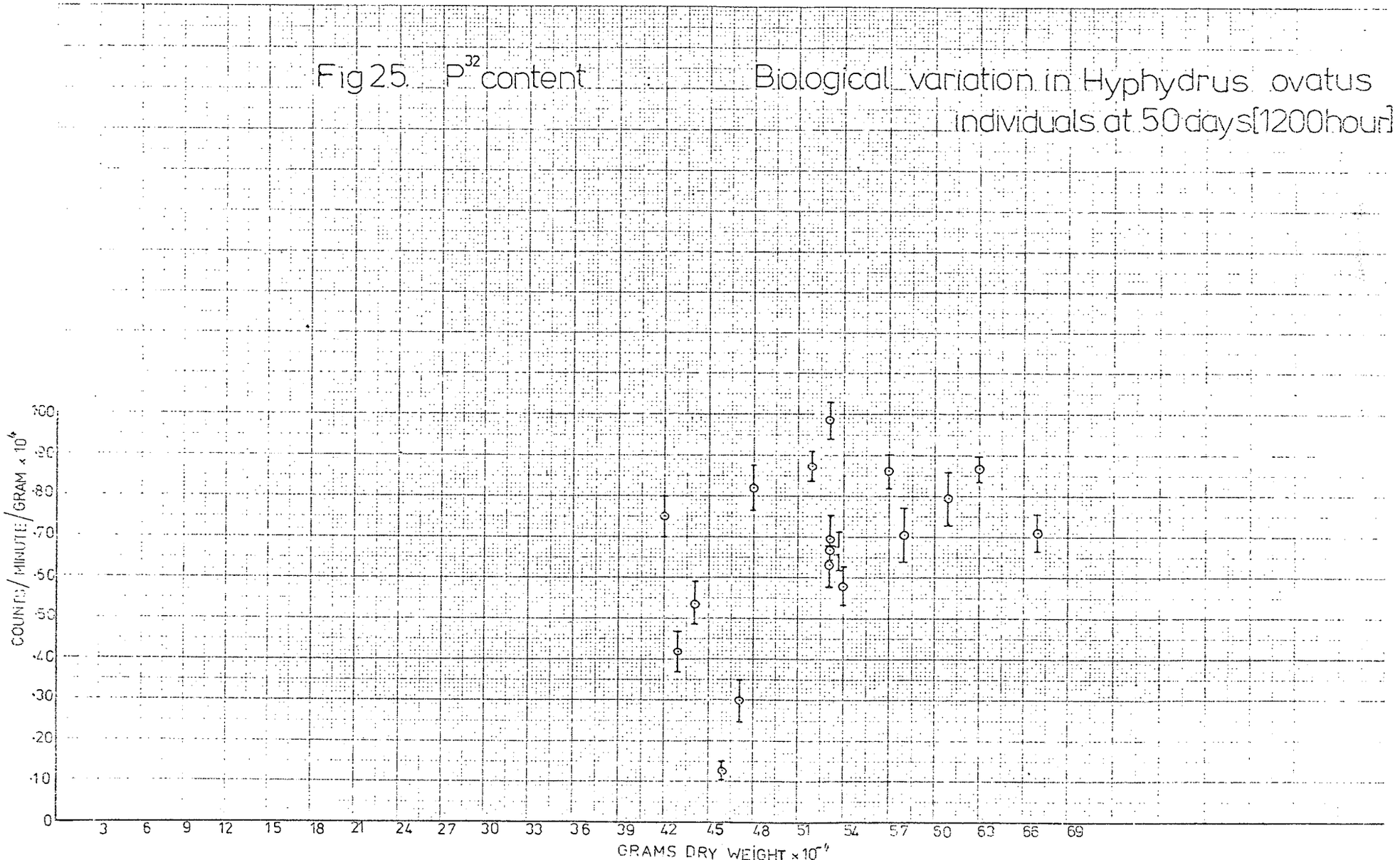


Fig 26. Limnophilus stigma P^{32} uptake/loss

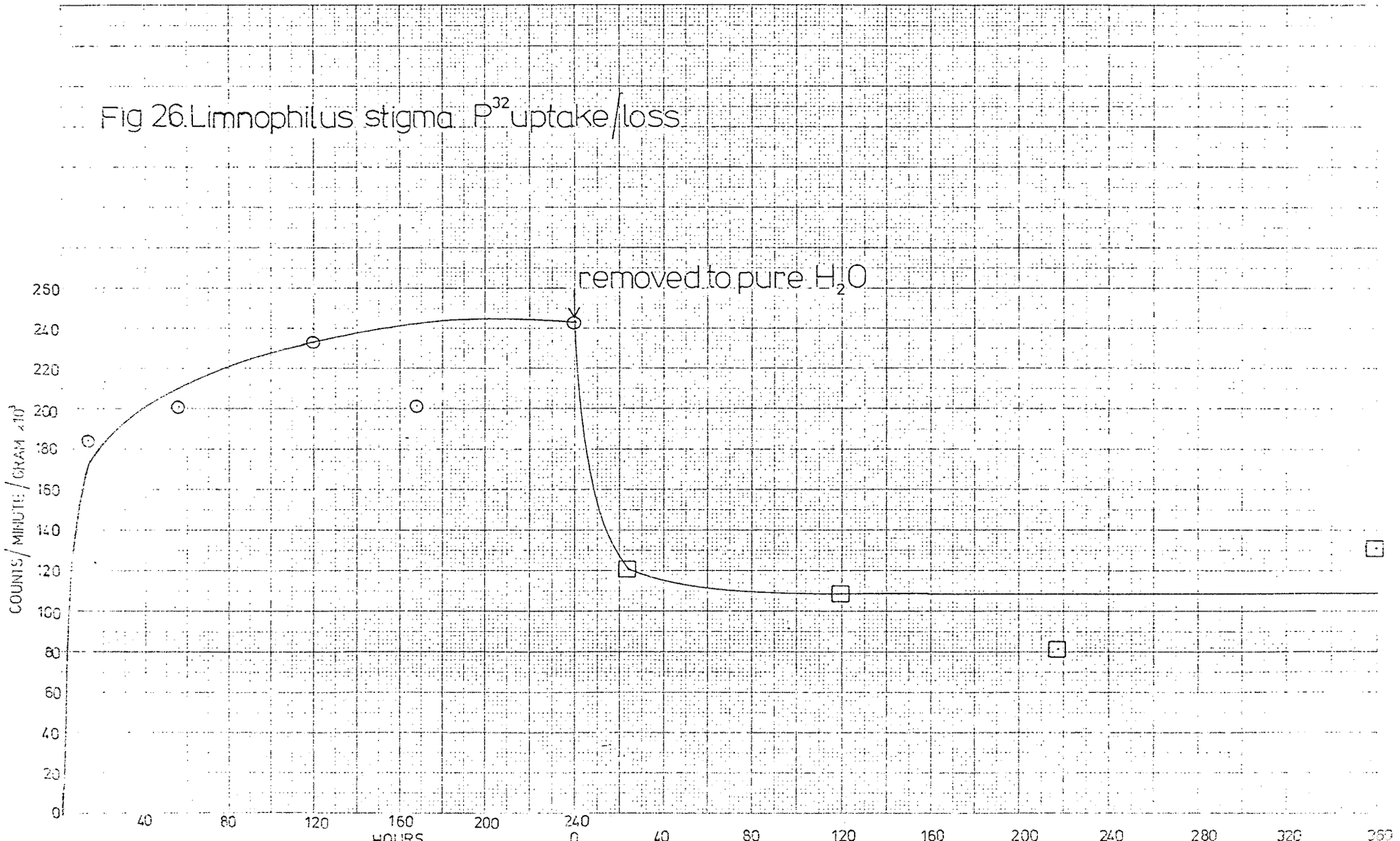
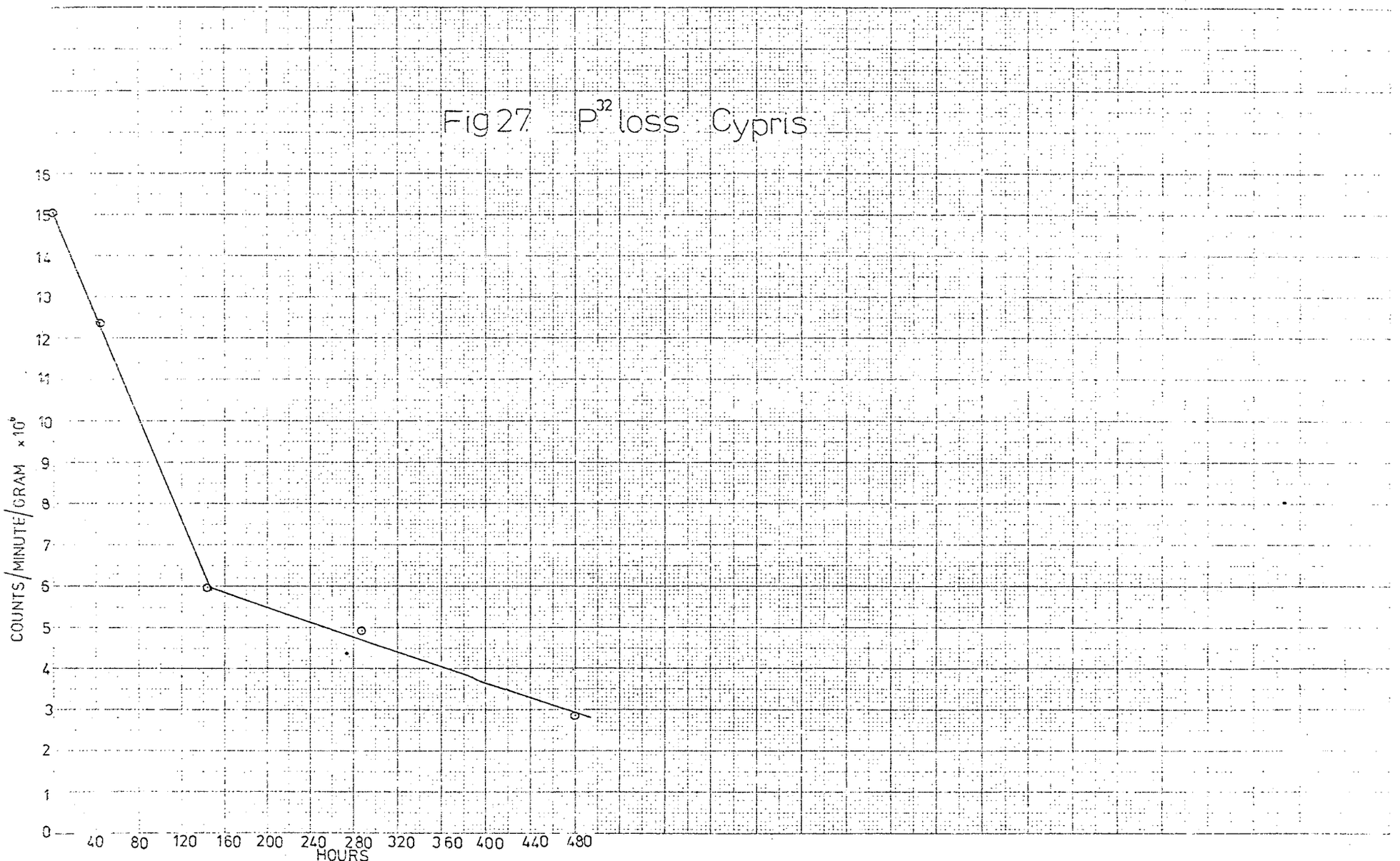


Fig 27 P^{32} loss : Cypris



TABLES

TABLE I ESTIMATED P^{32} DISTRIBUTION IN THE VARIOUS COMMUNITY FRACTIONS OF THE AQUARIUM MICROCOSM AFTER CORRECTING FOR THE EFFECTS OF SAMPLING AND NATURAL PREDATION

Activity Fraction (Total c.p.m.)	Millipore Fraction$_1$		Filtered Water$_2$		Tank Water (Whole 1+2)		Glass Sidewalls		Mud		Spirogyra		Callitriche		Animals		Deficit	
Time (Hours)	Net Corrected c.p.m.	% Total Tank P^{32}	Net Corrected c.p.m.	% Total Tank P^{32}	Net Corrected c.p.m.	% Total Tank P^{32}	Net Corrected c.p.m.	% Total Tank P^{32}	Net Corrected c.p.m.	% Total Tank P^{32}	Net Corrected c.p.m.	% Total Tank P^{32}	Net Corrected c.p.m.	% Total Tank P^{32}	Net Corrected c.p.m.	% Total Tank P^{32}	Net Corrected c.p.m.	% Total Tank P^{32}
1	$45.13x10^6$	52.15	$14.84x10^6$	17.15	$59.97x10^6$	69.30	$1.56x10^6$	1.80	$.59x10^6$	.74	$11.50x10^6$	13.29	$9.88x10^6$	11.42	$2.69x10^6$	3.11	$.5025x10^6$	.58
7.5	$28.16x10^6$	32.62	$7.80x10^6$	9.04	$35.96x10^6$	41.66	$4.27x10^6$	4.95	$4.42x10^6$	5.24	$12.80x10^6$	14.83	$14.90x10^6$	17.26	$6.20x10^6$	7.18	$7.66x10^6$	8.88
22	$15.67x10^6$	18.26	$1.98x10^6$	2.31	$17.65x10^6$	20.57	$4.78x10^6$	5.57	$4.84x10^6$	5.64	$14.40x10^6$	16.78	$19.20x10^6$	22.38	$10.29x10^6$	11.99	$14.59x10^6$	17.07
53	$7.39x10^6$	8.66	$0.44x10^6$	0.52	$7.83x10^6$	9.18	$4.78x10^6$	5.61	$5.14x10^6$	6.03	$17.00x10^6$	19.94	$20.10x10^6$	23.57	$12.80x10^6$	15.01	$17.62x10^6$	20.66
72	$6.14x10^6$	7.2	$0.36x10^6$	0.42	$6.50x10^6$	7.62	$4.76x10^6$	5.58	$6.03x10^6$	7.07	-	-	$20.50x10^6$	24.04	$12.30x10^6$	14.43	$35.18x10^6$	41.26
94	$4.37x10^6$	5.13	$0.44x10^6$	0.52	$4.81x10^6$	5.65	$4.74x10^6$	5.56	$6.96x10^6$	8.17	-	-	$20.80x10^6$	24.42	$12.97x10^6$	15.22	$34.92x10^6$	40.98
197	$2.10x10^6$	2.50	$0.51x10^6$	0.61	$2.61x10^6$	3.11	$3.97x10^6$	4.73	$20.79x10^6$	24.75	-	-	$19.80x10^6$	23.57	$12.67x10^6$	15.08	$24.17x10^6$	28.77
364	$1.69x10^6$	2.01	$0.41x10^6$	0.49	$2.08x10^6$	2.50	$2.90x10^6$	3.48	$39.46x10^6$	47.34	-	-	$13.60x10^6$	16.31	$8.42x10^6$	10.10	$16.91x10^6$	20.13
535	$1.33x10^6$	1.61	$0.29x10^6$	0.35	$1.62x10^6$	1.96	$1.53x10^6$	1.85	$43.14x10^6$	52.18	-	-	$4.91x10^6$	5.94	$6.90x10^6$	8.35	$24.58x10^6$	29.73
864	$0.97x10^6$	1.19	$0.42x10^6$	0.51	$1.39x10^6$	1.70	$1.13x10^6$	1.38	$40.97x10^6$	49.89	-	-	-	-	$3.75x10^6$	4.57	$34.87x10^6$	42.47
1200	$0.65x10^6$	0.79	$0.49x10^6$	0.60	$1.14x10^6$	1.39	$1.46x10^6$	1.78	$37.83x10^6$	46.07	-	-	-	-	$.79x10^6$	0.96	$40.89x10^6$	49.80

P^{32} DISTRIBUTION IN THE VARIOUS COMMUNITY FRACTIONS OF THE AQUARIUM MICROCOSM ASSUMING A CONSTANT BIOMASS FROM ZERO TIME ONWARDS

Community Fraction (Total c.p.m.)	Millipore Fraction$_1$		Filtered Water$_2$		Tank Water (Whole 1+2)		Glass Sidewalls		Mud		Spirogyra		Callitriche		Animals		Deficit	
Time (hours)	Net Corrected c.p.m.	% Total Tank P^{32}	Net Corrected c.p.m.	% Total Tank P^{32}	Net Corrected c.p.m.	% Total Tank P^{32}	Net Corrected c.p.m.	% Total Tank P^{32}	Net Corrected c.p.m.	% Total Tank P^{32}	Net Corrected c.p.m.	% Total Tank P^{32}	Net Corrected c.p.m.	% Total Tank P^{32}	Net Corrected c.p.m.	% Total Tank P^{32}	Net Corrected c.p.m.	% Total Tank P^{32}
1	45.13×10^6	52.05	14.84×10^6	17.11	59.97×10^6	69.16	1.57×10^6	1.81	$.59\times10^6$	00.68	11.56×10^6	13.33	9.90×10^6	11.42	2.71×10^5	3.13	$.41\times10^5$	0.47
7.5	28.16×10^6	32.48	7.80×10^6	8.99	35.96×10^6	41.47	4.34×10^6	5.01	4.42×10^6	5.10	13.21×10^6	15.23	14.97×10^6	17.26	6.94×10^6	8.00	6.88×10^6	7.93
22	15.67×10^6	18.07	1.98×10^6	2.28	17.65×10^6	20.35	4.87×10^6	5.62	4.84×10^6	5.58	16.80×10^6	19.37	19.42×10^6	22.39	13.10×10^6	15.10	10.04×10^6	11.58
53	7.39×10^6	8.52	0.44×10^6	0.51	7.83×10^6	9.03	4.95×10^6	5.71	5.14×10^6	5.93	22.44×10^6	25.88	20.36×10^6	23.48	17.65×10^6	20.35	8.34×10^6	9.62
72	6.14×10^6	7.09	0.36×10^6	0.42	6.50×10^6	7.51	4.93×10^6	5.69	6.03×10^6	6.95	-	-	20.87×10^6	24.07	17.86×10^6	20.60	30.53×10^6	35.20
94	4.37×10^6	5.04	0.44×10^6	0.51	4.81×10^6	5.55	4.90×10^6	5.65	6.96×10^6	8.03	-	-	21.19×10^6	24.44	18.21×10^6	21.00	30.65×10^6	35.34
197	2.10×10^6	2.42	0.51×10^6	0.59	2.61×10^6	3.01	4.22×10^6	4.86	20.79×10^6	23.97	-	-	20.68×10^6	23.85	18.43×10^6	21.25	20.00×10^6	23.06
364	1.67×10^6	1.91	0.41×10^6	0.47	2.08×10^6	2.38	3.13×10^6	3.61	39.46×10^6	45.50	-	-	14.50×10^6	16.72	16.06×10^6	18.52	11.51×10^6	13.27
555	1.33×10^6	1.54	0.29×10^6	0.33	1.62×10^6	1.87	1.67×10^6	1.93	43.14×10^6	49.75	-	-	8.12×10^6	9.36	14.92×10^6	17.20	17.25×10^6	19.89
864	0.97×10^6	1.12	0.42×10^6	0.48	1.39×10^6	1.60	1.23×10^6	1.42	40.97×10^6	47.25	-	-	-	-	12.36×10^6	14.25	30.77×10^6	35.48
1200	0.65×10^6	0.75	0.49×10^6	0.57	1.14×10^6	1.32	1.62×10^6	1.87	37.83×10^6	43.62	-	-	-	-	9.55×10^6	11.01	36.58×10^6	42.18

Table II

P^{32} CONTENT OF 10ml SAMPLES OF UNFILTERED AQUARIUM WATER

Sample Taken at (hours)	Background Count c.p.m.	Gross Sample Count c.p.m.	Nett Sample Count (less background) c.p.m.	Total Corrected Counts at Zero Time c.p.m.	Counts/Minute/Ml (gm) H_2O
1	170.00±2.08	660.32±5.43	490.32±5.81	4903.20±58.10	490.00±5.81
6.25	26.00± .82	360.17±4.09	334.17±4.17	3375.40±42.20	337.50±4.22
22	27.60± .87	166.87± .53	139.21±1.02	1443.10±10.60	144.31±1.06
51.5	15.66± .50	73.46±1.71	57.80±1.78	645.70±19.90	64.57±1.99
72	18.89± .60	68.31±1.40	49.42±1.52	531.40±16.30	53.14±1.63
94	15.84± .49	49.67±1.02	33.83±1.13	393.40±13.10	39.34±1.31
197	15.57± .49	30.74± .37	15.17± .60	213.70± 8.40	21.37± .84
364	14.89± .47	23.12± .37	8.23± .60	169.70±12.30	16.97±1.23
555	24.83± .02	29.44± .30	4.61± .30	132.60± 8.70	13.26± .87
1200*	54.37±1.00	71.48±1.36	17.11±1.69	929.90± 6.90	9.29± .69

* Sample consisted of 100 mls evaporated to 10 ml volume

Table III

P^{32} CONTENT OF 10ml SAMPLES OF AQUARIUM WATER FILTERED THROUGH MILLIPORE PAPERS (pore size = 0.45u)

Sample Taken at (hours)	Background Count c.p.m.	Gross Sample Count c.p.m.	Nett Sample Count (less background) c.p.m.	Total Corrected Counts at Zero Time c.p.m.	Counts/Minute/Ml H_2O
1	48.33± .88	169.69±1.51	121.36±1.75	1213.60±17.50	121.13±1.20
6.25	29.71± .90	92.90±2.08	63.19±2.26	638.00±22.80	63.80±2.30
22	25.67± .88	41.34± .23	15.67± .91	162.00± 9.50	16.20±1.00
51.5	17.85± .05	21.05± .16	3.20± .16	36.00± 1.80	3.60± .20
72	19.72± .05	22.43± .10	2.71± .11	29.00± 1.20	2.90± .10
94	18.00± .03	21.00± .15	3.00± .16	36.00± 1.90	3.60± .20
197	17.20± .31	20.17± .91	2.97± .97	42.00±13.60	4.20±1.40
364	-	-	-	-	-
555*	20.89±1.13	25.58± .15	4.69±1.15	120.20±29.40	2.40± .60
12000**	28.77± .91	36.07±1.14	7.30±1.46	400.10±79.90	4.0 ± .80

* Sample consisted of 50 mls evaporated to 10 ml volume

** Sample consisted of 100 mls evaporated to 10 ml volume

Table IV

P^{32} CONTENT OF MILLIPORE FILTER PAPERS **

Sample Taken at (hours)	Background Count c.p.m.	Gross Sample Count c.p.m.	Nett Sample Count (less background) c.p.m.	Total Corrected Counts at Zero Time c.p.m.	Counts/ Minute/ml Fraction	Sample Weight (gms dry)	Total Tank Biomass (gms dry)	Counts/ Minute/Gram
1	36.64±1.86	1709.98±15.69	1673.33±15.76	18800.00±178.00	376.00±3.60	.0009	2.2015	20.48×10^6
6.5	17.33± .55	1219.66± 8.03	1202.33± 8.05	13509.00± 91.00	270.20±1.80	.0008	1.9569	17.11×10^6
22	12.66± .40	496.32± 9.86	483.65± 9.87	6440.00±131.00	129.00±2.60	.0009	2.2015	7.12×10^6
51.5	16.71± .53	206.66± 3.17	189.95± 3.22	3040.00± 52.00	60.78±1.00	.0006	1.4677	5.08×10^6
72	13.46± .43	108.96± 1.31	210.09± 3.02	2977.00± 43.00	59.60± .86	.0006	1.4677	4.18×10^6
197	15.10± .48	33.81± .68	18.71± .83	849.00± 68.00	17.00± .80	.0005	1.1007	1.91×10^6
364	23.28± .74	46.01± 1.18	22.73± 1.39	689.00± 42.00	13.80± .80	.0003	.7339	2.29×10^6
555	24.77± .78	34.96± .18	10.20± .80	531.00± 42.00	10.60± .80	.0002	.4892	2.72×10^6
1200*	25.15± .80	30.54± .30	5.39± .85	980.00±155.00	9.80±1.60	.0002	.2446	1.77×10^6

** Represents the P^{32} content of solid matter suspended in 50 ml aliquots of aquarium water with a particle size in excess of .45 u

* 100 mls of filtered aquarium water were used here

Table V

P^{32} CONTENT OF GLASS SAMPLE PLATES AT VARIOUS TIMES DURING AQUARIUM MICROCOSM EXPERIMENT

Sample taken at (hrs)	Sample Area (cm^2)	Background Count (c.p.m.)	Gross Sample Count (c.p.m.)	Net Sample Count (less background) c.p.m.	Total Corrected Sample Count (c.p.m.) (x10)	Sample Weight (gms)	Total Tank Biomass (gms)	c.p.m./gm Biomass (x10)	Total Counts/ Tank (x10)
1.5	120	18.50± .59	80.24± .93	61.74±1.10	1,894± 33.75	.0050	.62193* .61693**	378,800	235,553
7.5	120	18.72± .59	151.96±1.37	133.24±1.49	3,488± 39.00	.0071	.88315* .86896**	491,268	433,862
23	135	19.30± .61	202.50±2.30	183.20±2.38	4,438± 57.66	.0092	1.01720* .99165**	482,391	490,677
53	135	16.83± .53	202.35±2.16	185.52±2.23	4,474± 53.71	.0108	1.19410* 1.15331**	414,259	494,669
125	195	13.22± .37	134.85± .55	121.63± .67	6,362± 34.89	.0230	1.76055* 1.67740**	276,609	486,962
168	195	14.25± .45	233.74±7.39	219.49±7.40	5,768±194.60	.0343	2.62551* 2.4672 **	168,163	441,499
363	180	15.84± .05	153.50±3.07	137.66±3.07	3,775±110.00	.0244	2.02336* 1.87696**	154,713	312,998
537	180	34.76±1.10	80.73±1.06	45.97±1.53	2,040± 67.70	.0270	2.23900* 2.04996**	75,556	169,144
912	195	26.00± .60	70.25±1.57	44.25±1.68	1,521± 57.79	.0286	2.18921* 1.97581**	53,181	116,417
1200	975	34.29±1.08	123.91± .90	89.63±1.41	10,581±166.50	.2171	3.3236 * 2.78253**	48,738	161,982

** Biomass estimate corrected for effect of progressive sample removal

* Biomass estimate as unaffected by sample removal (i.e. shows the true trend of biomass fluctuation in the tank)

Table VI

P^{32} CONTENT OF MUD SAMPLES IN THE AQUARIUM MICROCOSM

Time (Hours)	Sample Volume (cm^3)	Sample Weight (gms)	Total Corrected Counts (c.p.m.)	Counts/Minute/ Gram	Total c.p.m. in Tank Mud
9	30.0	18.41	8,140±18	442.5	5.31×10^6
22	37.8	22.25	8,964±34	403.3	4.84×10^6
53	48.9	32.13	13,770±33	428.5	5.14×10^6
96	37.8	28.21	16,550±67	586.8	7.04×10^6
174	56.7	39.57	44,040±181	1113.1	13.36×10^6
370	33.9	17.67	60,170±377	3405.3	40.88×10^6
557	56.7	35.44	43,170±108	1218.0	14.62×10^6
1200	183.9	97.77	308,110±143	3151.5	37.83×10^6

Table VII

P^{32} CONTENT OF SEDIMENT SAMPLES IN THE AQUARIUM MICROCOSM

Sample Period (Days)	Area Sampled (cm^2)	Sample Weight (gms)	Total Corrected Counts (c.p.m.)	Total Weight Sedimenting out in Tank (gms)	Rate of Sedimentation (gms/hr)	Total P^{32} Sedimenting into Tank Mud (c.p.m.)	Average Rate of Sedimentation during Sample per hour (c.p.m.)	Sediment c/m/gm*
0-7	10.18	.048	$40.43 \times 10^3 \pm 0.15 \times 10^3$	20.60	.123	17.34×10^6	10.32×10^4	$.842 \times 10^6$
7-36	10.18	.132	$41.37 \times 10^3 \pm 0.74 \times 10^3$	56.71	.082	17.75×10^6	2.55×10^4	$.323 \times 10^6$
36-50	10.18	.063	$13.15 \times 10^3 \pm 0.21 \times 10^3$	27.02	.080	5.64×10^6	1.68×10^4	$.209 \times 10^6$

Total weight sedimenting into mud after 50 days — 104.33

Total P^{32} sedimenting into mud after 50 days — 40.73×10^6

* Average figure for whole sample period

Table VIII

Table IX

CONCENTRATION RATIOS RECORDED IN MICROCOSM EXPERIMENT

Species/ Community Fraction	Concentration Ratio at Time of Maximum Activity Density	Time of Maximum Activity Density (hours)	Maximum Concentration Ratio Recorded	Time of Maximum Concentration Ratio (hours)
Millipore Fraction	168,819	1	1,447,817	72
Sidewall Plates	81,851	7.5	1,275,862	72
Sediment	215,892	252	293,103	72
Mud	896	370	1,387	556
Spirogyra	623,960*	53	623,960	53
Callitriche	157,339	96	157,339	96
Dytiscus (larvae)	1,215,547	168	1,215,547	168
Dytiscus (adult)	268,601	528	268,601	528
Gasterosteus (adult)	1,390,000*	1200	1,390,000	530
Gasterosteus (fry)	3,267,060*	530	3,267,060	530
Nepa	98,855	168	98,855	168
Notonecta	127,208	168	127,208	168
Hyphydrus	239,086	53	262,500	555
Agabus	178,390	252	245,833	555
Sialis	231,453*	1200	231,453	1200
Enallagma	503,950	363	503,950	363
Gerris	221,731	864	221,731	864
Erpobdella	181,667*	1200	181,667	1200
Polycelis	65,000	864	82,000	555
Hydracarina	570	14	2,100	22
Hydrobius	240,195	216	321,000	555
Limnaea				
Body	540,740	240	925,918	168
Shell	39,286	103	76,717	119
Planorbis	276,557	363	276,557	363
Limnaea Eggs	662,110*	529	662,110	529
Rana Tadpoles	1,206,733	53	1,474,462	72
Limnophilus stigma	315,000*	864	316,000	555
Leptocerus	322,000*	1200	322,000	1200
Limnophilus xanthodes	560,000	612	599,000	555
Corixa	20,185	8	358,333	555
Daphnia	2,202,343*	53	2,202,343	53

* peak levels of activity density were not reached in these species during the time of the experiment; concentration ratios were therefore based on peak values recorded.

(a) RATE VALUES FOR P^{32} UPTAKE BY THE VARIOUS COMMUNITY FRACTIONS IN THE AQUARIUM MICROCOSM

	Gross Uptake Rate per Hour (r_1)	Activity Uptake Rate uc/gm/hr (r_2)	Turnover Rate (r_4)	Maximum Activity Density (uc/gm dry weight)	Turnover Time (Hours) $\frac{1}{r_4}$
FILTERED WATER	-	-	.829	-	1.21
MILLIPORE FRACTION	.5200	9.2300	1.000	9.23	1.00
SIDEWALL FRACTION	.0190	1.1400	.510	2.21	1.99
SEDIMENT	.0021	.4730	.010	.38	100.00
MUD (BIOLOGICAL UPTAKE)	.0171	-	.130	-	7.70
MUD (VIA SEDIMENT)	1.0000	3.8×10^{-6}	-	-	-
MUD (VIA DEATH AND DECAY)	.0037	3.5×10^{-6}	-	-	-
MUD (ALL ROUTES)	.0050	1.6×10^{-5}	.0053	.003	190.00
CALLITRICHE	.1142	.1186	.467	.25	2.14
SPIROGYRA	.1333	.5207	.511	1.01	1.96
RANA TADPOLES	.1625	.770	.389	1.96	2.60
CORIXA	.0027	.065	.125	.52	8.00
LIMNOPHILUS STIGMA	.0289	.00175	.004	.45	250.00
LIMNOPHILUS XANTHODES	.0026	.0188	.029	.60	34.50
LIMNEA BODY	.0033	.0081	.013	.63(1.20)*	76.90
LIMNEA SHELL	.0008	.0054	-	-	-
LIMNEA EGGS	.0004	.0122	.017	.72	58.80
PLANORBIS (BODY AND SHELL)	.0004	.0037	.011	.32	90.90
HYDROBIUS	.00005	.0023	.005	.45	200.00
LEPTOCERUS AND TRIANODES	.0028	.0026	.004	.60	250.00
DAPHNIA	.0192	.391	.113	3.47	8.85
GASTEROSTEUS (ADULT)	.0066	.0184	.007	2.50	142.86
GASTEROSTEUS (FRY)	.0065	.0197	.006	3.54	166.67
DYTISCUS LARVA	.0051	.0092	.006	1.55	166.67
DYTISCUS ADULT	.00027	.00056	.002	.29	500.00
AGABUS	.00035	.00177	.006	.30	166.67
HYPHYDRUS	.00198	.0071	.019	.38	52.67
NOTONECTA	.00048	.0024	.015	.15	66.67
NEPA	.007	.0025	.020	.13	50.00
ENALLAGMA	.0066	.0033	.006	.60	166.67
SIALIS LARVAE	.00052	.00035	.001	.41	1000
ERPOBDELLA	.00370	.00023	.001	.33	1000
POLYCELIS	.00082	.00023	.002	.10	500
GERRIS	.00024	.00037	.001	.32	1000

* Maximum value reached by a single individual.

Table X

BIOMASS (DRY WEIGHT) ESTIMATES AT ZERO HOURS IN THE AQUARIUM MICROCOSM

	Average Weight per Individual (gms.)	Number at Zero Hours	Estimated Biomass (gms)	Number Removed After 50 Days	Biomass Removed (gms.)	% of Total Biomass Removed	Number per Sample
PLANORBIS	.00640	159	1.0286	49	.3032	29.80	5 - 8
LIMNEA (BODY)	.02482	170	4.2187	36	.8934	21.20	3 - 5
LEPTOCERUS	.00291	110	.3196	34	.0988	30.90	3 - 5
LIMNOPHILUS STIGMA	.01969	75	1.4765	16	.2953	20.00	2
LIMNOPHILUS XANTHODES	.00865	70	.6055	14	.1211	20.00	2
TRIAENODES	.00186	250	.4638	8	.0149	3.50	4
RANA TADPOLES	.00438	250	1.0945	37	.0846	7.70	2 - 8
CORIXA	.00242	350	.8484	29	.0703	8.20	4 - 5
HYDROBIUS	.01035	10	.1035	10	.0317	30.60	1
CHORIS	.00212	23	.0488	19	.0403	82.60	1 - 3
ENALLAGMA	.00386	150	.5786 (.8)	35	.1350	23.30	4 - 5
POLYCELIS	.00119	120	.1428	50	.0595	41.70	4 - 30
HYPHYDRUS	.00538	75	.4036	60	.3229	80.00	2 - 23
AGABUS	.01500	12	.1800	8	.1200	66.00	2 - 3
DYTISCUS (LARVAE)	.06026	9	.5423	9	.5423	100.00	1 - 3
DYTISCUS (ADULT)	.07322	6	.4393	6	.4393	100.00	1 - 2
NOTONECTA	.02386	8	.1909	8	.1909	100.00	1 - 3
NEPA	.03053	14	.4274	13	.3969	83.40	1 - 2
ERPOBDELLA	.01046	60	.6278	57	.5964	81.40	1 - 30
HYDRACARINA	.00042	280	.1168	56	.0234	20.10	3 - 15
SIALIS	.00558	25	.1395	21	.1172	84.00	3 - 15
GASTEROSTEUS (ADULT)	.03834	10	.3834	9	.3451	90.00	1
GASTEROSTEUS (FRY)	.00185	70	.1296	18	.0334	25.70	1 - 3
LIMNEA (SHELL)	.03486	170	5.9263	36	1.255	20.00	3 - 5
LIMNEA (EGGS)	-	-	1.2940	-	.1294	10.00	-
DAPHNIA	.0000425	-	.5000	-	.0017	.0033	10 - 15

Table X

(a) P^{32} UPTAKE WITH TIME BY SPIROGYRA SPP. IN THE AQUARIUM MICROCOSM

Sample Taken at (hours)	Sample Weight (gms dry)	Background Count c.p.m.	Gross Sample Count c.p.m.	Nett Sample Count (less background) c.p.m.	Counts/Minute/Gram at Zero Time
1	.0788	14.07±1.07	2169 ±20.26	2154.99±20.29	$1.16 \times 10^6 \pm 10,880$
7.5	.0359	13.71±1.27	525.25± 6.93	511.54± 7.05	$1.33 \times 10^6 \pm 14,130$
26	.0583	14.80±1.39	1635.98±13.42	1621.18±13.49	$1.63 \times 10^6 \pm 13,560$
53	.0305	12.07± .95	1374.32±11.80	1362.25±11.84	$2.24 \times 10^6 \pm 19,495$

(b) P^{32} UPTAKE WITH TIME BY CALLITRICHE SPP. IN THE AQUARIUM MICROCOSM

1	.0571	16.43±1.71	317.10± 3.23	300.67± 3.66	$2.63 \times 10^5 \pm 3,200$
7.5	.0875	17.03±1.78	501.03± 4.11	484.00± 4.48	$3.98 \times 10^5 \pm 3,680$
22	.2390	16.52±1.12	784.10±10.67	767.58±10.72	$5.17 \times 10^5 \pm 7,220$
53	.0992	25.76±1.29	756.43±10.67	730.71±10.74	$5.42 \times 10^5 \pm 7,960$
96	.0900	31.31±1.68	1474.20±12.82	1442.89±12.93	$5.62 \times 10^5 \pm 5,030$
168	.0837	25.40± .90	1621.77± 8.27	1596.37± 8.32	$5.61 \times 10^5 \pm 2,920$
363	.0853	27.94± .99	981.91± 6.87	953.98± 6.94	$3.86 \times 10^5 \pm 2,810$
513	.0933	25.81±1.33	460.75± 4.84	434.94± 5.02	$2.16 \times 10^5 \pm 2,500$

Table XI

(a) P^{32} UPTAKE WITH TIME BY LIMNEA PEREGER (BODIES) IN THE AQUARIUM MICROCOSM

Sample Taken at (hours)	Sample Weight (gms dry)	Background Count c.p.m.	Gross Sample Count c.p.m.	Nett Sample Count (less background) c.p.m.	Counts/Minute/Gram at Zero Time	Average c.p.m./gm at Zero Time
1	.0345	15.26± .44	19.01± .56	3.76± .72	$.03 \times 10^5 \pm$ 580	$.04 \times 10^5 \pm$ 700
	.0167	15.65± .49	20.72± .61	5.06± .79	$.05 \times 10^5 \pm$ 830	
8	.0238	25.19±3.04	220.05± 3.85	194.85± 4.91	$1.97 \times 10^5 \pm$ 4,970	$1.19 \times 10^5 \pm$ 4,860
	.0117	24.02±2.13	44.64± 1.04	20.62± 2.37	$.42 \times 10^5 \pm$ 4,740	
24	.1115	29.05±1.73	1955.19± 8.60	1926.13± 8.78	$4.14 \times 10^5 \pm$ 1,890	
53	.0700	25.97±1.86	2133.96±49.70	2107.99±49.79	$7.21 \times 10^5 \pm$17,010	
103	.0710	25.48±1.61	2880.28±31.97	2854.80±32.01	$9.63 \times 10^5 \pm$10,800	
119	.0824	24.99±1.69	3476.58±28.85	3451.58±28.91	$1.41 \times 10^6 \pm$11,760	
168	.0132	25.44±1.27	1437.12±14.37	1411.68±14.43	$2.59 \times 10^6 \pm$26,010	
	.0285	27.65±1.38	1068.80±15.17	1041.15±15.24	$8.86 \times 10^5 \pm$12,930	$1.32 \times 10^6 \pm$11,680
	.0323	24.15± .79	1215.56± 3.77	1191.41± 3.85	$1.12 \times 10^6 \pm$ 3,640	
	.0206	28.27±1.41	483.75± 2.42	455.49± 2.79	$6.75 \times 10^5 \pm$ 4,150	
363	.0986	26.42±1.26	1763.00± 8.99	1736.73± 9.08	$1.32 \times 10^6 \pm$ 6,940	
530	.0917	27.44± .93	1109.96± 3.55	1082.53± 3.67	$1.31 \times 10^6 \pm$ 4,490	
864	.0795	31.69±1.05	811.12±11.36	779.44±11.40	$1.19 \times 10^6 \pm$17,500	
1200	.0972	29.57±1.89	922.22±18.08	892.65±18.17	$1.07 \times 10^6 \pm$21,300	

Table XII

(b) P^{32} UPTAKE WITH TIME BY LIMNEA PEREGER (SHELLS) IN THE AQUARIUM MICROCOSM

Sample Taken at (hours)	Sample Weight (gms dry)	Background Count c.p.m.	Gross Sample Count c.p.m.	Nett Sample Count (less background) c.p.m.	Counts/Minute/Gram at Zero Time	Average c.p.m./gm. at Zero Time
1	.0434	14.84± .86	42.33± .61	27.49± 1.06	$.11 \times 10^5 \pm$ 420	$.12 \times 10^5 \pm$ 660
	.0210	14.75± 1.16	30.97± .09	16.22± 1.12	$.13 \times 10^5 \pm$ 900	
8	.0371	115.44±10.27	295.34± 4.90	179.87±11.38	$1.17 \times 10^5 \pm 7,380$	$.78 \times 10^5 \pm 4,650$
	.0222	35.70± 1.29	73.00± 1.20	37.30± 1.76	$.40 \times 10^5 \pm 1,910$	
24	.1574	31.59± 1.31	223.52± .21	191.93± 1.32	$1.02 \times 10^5 \pm$ 700	
53	.1537	29.51± .92	199.41± 1.73	169.90± 1.96	$.97 \times 10^5 \pm 1,110$	
103	.0955	25.35± 1.37	469.21± 4.46	443.86± 4.66	$1.11 \times 10^5 \pm 1,170$	
119	.1372	27.52± 2.18	1253.59±13.41	1226.06±13.59	$2.14 \times 10^5 \pm 2,370$	
168	.0295	21.22± 2.91	77.74± 1.92	56.52± 3.50	$.48 \times 10^5 \pm 2,960$	
	.0315	18.66± .97	52.74± 1.18	34.08± 2.17	$.55 \times 10^5 \pm 3,480$	$.53 \times 10^5 \pm 3,020$
	.0360	21.17± .89	92.30± 1.19	71.13± 1.49	$.58 \times 10^5 \pm 1,200$	
	.0306	16.04± 2.82	53.25± 1.67	37.21± 3.27	$.50 \times 10^5 \pm 4,410$	
363	.1294	24.28± 1.08	213.94± 2.85	189.66± 3.05	$.95 \times 10^5 \pm 1,520$	
530	.1025	23.12± 1.07	71.12± 1.88	48.00± 2.16	$.29 \times 10^5 \pm 1,290$	
864	.1248	27.88± 1.39	149.92± 5.64	122.05± 5.87	$1.06 \times 10^5 \pm$ 510	
1200	.1032	30.95± 1.99	53.17± 2.33	22.22± 3.07	$.23 \times 10^5 \pm$ 320	

Table XII

P^{32} UPTAKE WITH TIME BY CORIXA SPP. IN THE AQUARIUM MICROCOSM

Sample Taken at (hours)	Sample Weight (gms dry)	Background Count c.p.m.	Gross Sample Count c.p.m.	Nett Sample Count (less background) c.p.m.	Counts/Minute/Gram at Zero Time
1	.0091	10.46± .83	35.61± 2.71	25.14± 2.83	$.84 \times 10^5 \pm$ 9,421
8	.0126	16.71± .53	1002.50±11.20	985.80±11.22	$1.15 \times 10^6 \pm$13,090
22	.0063	33.40±1.35	437.50± 8.80	404.09± 8.90	$1.14 \times 10^6 \pm$25,220
53	.0129	35.60±2.71	-	-	-
168	.0082	23.89±1.50	231.18± 5.60	207.29± 5.80	$9.41 \times 10^5 \pm$26,290
363	.0042	35.68±1.26	120.72± 1.37	85.04± 1.86	$7.78 \times 10^5 \pm$23,400
557	.0170	28.27±1.29	122.10± 3.05	93.82± 3.32	$8.54 \times 10^5 \pm$30,140

Table XIII

P^{32} UPTAKE WITH TIME BY RANA TADPOLES IN THE AQUARIUM MICROCOSM

Sample Taken at (hours)	Sample Weight (gms dry)	Background Count c.p.m.	Gross Sample Count c.p.m.	Nett Sample Count (less background) c.p.m.	Counts/Minute/Gram at Zero Time
1	.0129	16.02±1.59	590.97± 4.02	574.95± 4.32	$1.71 \times 10^6 \pm 12,500$
9	.0066	25.35±2.52	636.36± 5.80	611.01± 6.32	$2.56 \times 10^6 \pm 26,500$
24	.0060	16.81±1.29	324.17± 2.08	807.36± 2.45	$3.66 \times 10^6 \pm 6,180$
53	.0114	13.72±0.75	2486.84±19.15	2473.12±19.16	$4.34 \times 10^6 \pm 33,620$
168	.0327	13.88±0.69	6177.92±27.18	6164.04±27.19	$4.19 \times 10^6 \pm 18,480$
557	.0090	24.83±0.78	566.30± 3.17	531.47± 3.27	$2.89 \times 10^6 \pm 17,640$

Table XIV

(a) P^{32} UPTAKE WITH TIME BY LIMNOPHILUS XANTHODES IN THE AQUARIUM MICROCOSM

Sample Taken at (hours)	Sample Weight (gms dry)	Background Count c.p.m.	Gross Sample Count c.p.m.	Nett Sample Count (less background) c.p.m.	Counts/Minute/Gram at Zero Time
8	.0264	13.85±1.13	405.68± 5.48	391.83± 5.59	$3.34 \times 10^5 \pm$ 4,760
24	.0119	16.46±1.34	444.77±11.61	428.31±11.69	$8.09 \times 10^5 \pm$22,060
168	.0229	14.83± .47	480.77±20.91	465.94±20.92	$5.73 \times 10^5 \pm$21,240
363	.0104	28.21±1.20	337.41± 4.69	309.20± 4.84	$1.18 \times 10^6 \pm$16,160
513	.0128	24.18±1.21	538.88± 5.93	514.70± 6.05	$1.39 \times 10^6 \pm$16,210
864	.0200	30.04±1.15	385.66±10.07	355.62±10.13	$1.31 \times 10^6 \pm$37,200
1200	.0167	30.09± .98	204.18± 1.78	174.08± 2.03	$1.09 \times 10^6 \pm$12,660

(b) P^{32} UPTAKE WITH TIME BY LIMNOPHILUS STIGMA IN THE AQUARIUM MICROCOSM

22	.0477	26.80±1.54	160.70± 3.92	133.90± 4.21	$.62 \times 10^5 \pm$ 1,963
53	.0304	15.59±1.53	158.67± 1.10	143.08± 1.88	$1.05 \times 10^5 \pm$ 1,300
103	.0501	30.50±1.53	709.80± 9.16	679.3 ± 9.28	$3.99 \times 10^5 \pm$ 5,430
168	.0467	21.07±1.08	944.63± .23	923.56± 1.10	$4.39 \times 10^5 \pm$ 525
363	.0387	28.39±1.57	343.65± 2.27	315.26± 2.76	$4.93 \times 10^5 \pm$ 4,310
529	.0440	24.41±1.19	980.76± 8.24	956.55± 8.31	$7.34 \times 10^5 \pm$ 6,400
864	.0302	29.80±1.88	479.87±13.48	450.06±13.61	$1.01 \times 10^6 \pm$30,400

Table XV

(c) P^{32} UPTAKE WITH TIME BY LEPTOCERUS SPP. IN THE AQUARIUM MICROCOSM

Sample Taken at (hours)	Sample Weight (gms dry)	Background Count c.p.m.	Gross Sample Count c.p.m.	Nett Sample Count (less background) c.p.m.	Counts/Minute/Gram at Zero Time
1	.0088	14.80± .38	16.14± .26	1.34± .46	$.03 \times 10^5$
8	.0105	12.89± .18	30.48± .48	17.59± .51	$.34 \times 10^5$
22	.0153	14.82± .56	110.27±2.67	95.45±2.73	1.25×10^5
53	.0110	15.31± .14	125.29±1.41	109.98±1.41	1.99×10^5
119	.0090	14.18± .74	67.89± .12	53.7 ± .75	2.28×10^5
168	.0065	13.16±1.27	94.42±2.20	81.26±2.54	2.78×10^5
363	.0074	22.97±1.20	142.52± .93	119.56±1.52	5.53×10^5
513	.0087	26.24±1.31	186.68±2.99	160.44±3.27	7.04×10^5
864	.0125	27.89±1.04	197.92±2.39	170.02±2.61	9.45×10^5
1200	.0091	28.93±1.61	136.79±5.87	107.86±6.08	12.88×10^5

Table XV

(a) P^{32} UPTAKE WITH TIME BY LIMNAEA EGGS IN THE AQUARIUM MICROCOSM

Sample Taken at (hours)	Sample Weight (gms dry)	Background Count c.p.m.	Gross Sample Count c.p.m.	Nett Sample Count (less background) c.p.m.	Counts/Minute/Gram at Zero Time
1	.0108	16.88± .60	40.97± .92	24.10±1.09	$.27 \times 10^5 \pm$ 1,348
22	-	-	-	-	-
53	.0087	14.13±1.46	229.97±2.21	215.8 ±2.65	$3.29 \times 10^5 \pm$ 40,043
168	.0136	18.52± .93	338.98±6.17	320.46±6.24	$4.88 \times 10^5 \pm$ 9,510
363	.0066	25.75±1.45	196.73±1.48	170.98±1.85	$1.14 \times 10^6 \pm$ 12,230
529	.0025	23.80± .86	100.21±1.79	76.41±1.98	$1.59 \times 10^6 \pm$ 41,390

(b) P^{32} UPTAKE WITH TIME BY PLANORBIS ALBUS IN THE AQUARIUM MICROCOSM

1	.0191	12.65±1.17	62.43±1.21	49.78±1.69	$.36 \times 10^5 \pm$ 1,226
8	.0252	15.93± .90	127.63±1.74	111.70±1.96	$.65 \times 10^5 \pm$ 1,141
26	.0313	15.95±1.01	351.46±1.87	335.52±2.12	$1.65 \times 10^5 \pm$ 1,040
53	.0452	14.60± .86	894.02±3.30	879.42±3.41	$3.04 \times 10^5 \pm$ 1,180
103	.0412	14.90± .96	993.79±9.04	978.89±9.09	$4.66 \times 10^5 \pm$ 433
168	.0276	19.48± .97	702.34±8.36	682.86±8.41	$5.15 \times 10^5 \pm$ 6,350
363	.0412	28.84±1.72	521.41±4.38	492.57±4.70	$7.25 \times 10^5 \pm$ 6,920
530	.0372	24.52±1.40	367.60±5.18	343.08±5.37	$5.27 \times 10^5 \pm$ 8,250
864	.0352	23.18±1.22	325.60±4.26	302.16±4.43	$5.96 \times 10^5 \pm$ 8,400
1200	.0114	27.60±1.38	70.46±1.30	42.86±1.90	$3.92 \times 10^5 \pm$ 2,100

Table XVI

(a) P^{32} UPTAKE WITH TIME BY HYPHYDRUS OVATUS IN THE AQUARIUM MICROCOSM

Sample Taken at (hours)	Sample Weight (gms dry)	Background Count c.p.m.	Gross Sample Count c.p.m.	Nett Sample Count (less background) c.p.m.	Counts/Minute/Gram at Zero Time
1	.0130	26.39± .92	73.21± .60	46.82± 1.09	1.74×10^5± 4,074
8	.0190	25.83±1.29	155.62±10.23	129.79±10.32	2.91×10^5±23,100
22	.0155	25.54±1.27	111.29± 1.90	85.75± 2.29	3.04×10^5± 8,100
53	.0118	22.81± .83	333.85± 3.41	311.04± 3.50	8.37×10^5± 9,430
168	.0135	23.99± .88	358.27± 4.19	334.28± 4.28	7.86×10^5±10,070
363	.0129	32.50±1.14	288.06± 3.83	255.60± 3.99	7.08×10^5±11,070
530	.0114	26.85±1.49	203.44± .48	176.58± 1.57	5.67×10^5± 5,300
864	.0152	20.77±1.70	136.61± 7.59	115.79± 7.78	5.29×10^5±35,530
1200	.1953	28.80±1.54	757.58± 7.58	728.77± 7.23	5.83×10^5± 9,900

(b) P^{32} UPTAKE WITH TIME BY AGABUS SP. IN THE AQUARIUM MICROCOSM

168	.0480	29.70± .94	1241.20±12.12	1211.50±12.15	6.64×10^5± 6,660
555	.0420	22.11± .70	501.52± 5.02	479.41± 5.07	5.95×10^5± 6,290
1200	.030	31.75±1.00	120.80± 3.82	89.05± 3.95	4.93×10^5±21,860

Table XVII

(c) P^{32} UPTAKE WITH TIME BY NEPA CINEREA IN THE AQUARIUM MICROCOSM

Sample Taken at (hours)	Sample Weight (gms dry)	Background Count c.p.m.	Gross Sample Count c.p.m.	Nett Sample Count (less background) c.p.m.	Counts/Minute/Gram at Zero Time
8	.0320	27.35±1.37	54.72± .27	27.37±1.39	$.24 \times 10^5 \pm$ 1,210
26	.0509	25.85±1.22	494.73±1.53	468.88±1.96	$2.56 \times 10^5 \pm$ 1,070
53	.0600	28.24±1.03	586.55±3.29	558.30±3.44	$2.59 \times 10^5 \pm$ 1,590
168	.0511	21.09±1.06	557.15± .72	536.10±1.28	$2.79 \times 10^5 \pm$ 670
363	.0534	26.95±1.53	179.77±3.67	152.83±3.97	$1.22 \times 10^5 \pm$ 3,170
513	.0412	25.02±1.21	158.20±2.50	133.18±2.78	$1.96 \times 10^5 \pm$ 4,800
864	.1083	26.60±..17	313.97±4.65	287.37±4.79	$1.84 \times 10^5 \pm$ 3,060

Table XVII

(a) P^{32} UPTAKE WITH TIME BY DYTISCUS MARGINALIS LARVAE IN THE AQUARIUM MICROCOSM

Sample Taken at (hours)	Sample Weight (gms dry)	Background Count c.p.m.	Gross Sample Count c.p.m.	Nett Sample Count (less background) c.p.m.	Counts/Minute/Gram at Zero Time
8	.1146	23.42±1.18	263.39± 4.77	239.97± 4.91	$.63 \times 10^5 \pm$ 1,300
22	.0326	26.22± .83	597.27± .93	571.06± 1.25	$4.49 \times 10^5 \pm$ 980
53	.0696	24.60±1.23	2979.20±12.94	2954.60±12.99	$1.12 \times 10^6 \pm$ 4,900
168	.0329	29.48±1.47	4558.91± 4.53	4527.96± 4.76	$3.44 \times 10^6 \pm$ 3,620
363	.1433	26.64±1.01	4617.89±22.35	4590.24±22.37	$1.09 \times 10^6 \pm$ 5,290
513	.1493	25.85±1.62	1011.59±11.31	985.74±11.42	$2.50 \times 10^5 \pm$ 2,900

(b) P^{32} UPTAKE WITH TIME BY CASTE DYTISCUS LARVAE SKINS IN THE AQUARIUM MICROCOSM

26	.0030	26.38± .83	62.01± 1.96	35.63± 2.13	$.82 \times 10^6 \pm$ 49,280
119	.0013	28.43± .90	86.50± 2.68	58.08± 2.82	$3.10 \times 10^6 \pm$ 151,050

Table XVIII

(a) P^{32} UPTAKE WITH TIME BY GASTEROSTEUS ACULEATUS ADULTS IN THE AQUARIUM MICROCOSM

Sample Taken at (hours)	Sample Weight (gms dry)	Background Count c.p.m.	Gross Sample Count c.p.m.	Nett Sample Count (less background) c.p.m.	Counts/Minute/Gram at Zero Time
8	.0476	23.62±1.06	477.16± 3.15	453.55± 3.32	$4.14 \times 10^5 \pm$ 3,030
26	.0498	25.07±1.12	2577.26±13.58	2552.19±13.63	$1.35 \times 10^6 \pm$ 8,150
53	.0303	24.94±1.03	2516.06±11.60	2491.20±11.65	$2.16 \times 10^6 \pm$10,120
168	.0794	27.23±1.36	3183.87±10.07	3156.64±10.16	$2.09 \times 10^6 \pm$ 6,730
	.0228	26.47±1.68	1062.62± 8.98	1036.15± 9.14	$2.59 \times 10^6 \pm$25,000
363	.0213	23.79±1.40	1344.58±10.31	1320.79±10.41	$2.22 \times 10^6 \pm$17,000
530	.0460	30.69±1.69	4329.77±16.02	4299.08±16.11	$3.34 \times 10^6 \pm$10,000
864	.0503	22.71±1.14	2140.18±15.13	2117.47±15.18	$4.41 \times 10^6 \pm$30,800
1200	.0381	29.44±1.78	1895.73±12.78	1866.29±12.90	$5.56 \times 10^6 \pm$37,400

(b) P^{32} UPTAKE WITH TIME BY GASTEROSTEUS ACULEATUS FRY IN THE AQUARIUM MICROCOSM

1	.0025	15.63± .49	37.62± .75	21.99± .90	$1.93 \times 10^5 \pm$ 9,900
9	.0029	16.98±1.29	94.30± 1.24	77.33± 1.79	$6.49 \times 10^5 \pm$15,010
26	.0083	16.21±1.52	1101.58± 9.91	1085.37±10.03	$1.53 \times 10^6 \pm$14,060
53	.0017	27.21± .76	242.28± 2.46	215.06± 2.58	$2.32 \times 10^6 \pm$27,810
168	.0080	24.42±1.22	2377.80±25.06	2353.39±25.09	$6.13 \times 10^6 \pm$65,340
363	.0015	27.94± .91	308.05± 3.92	280.05± 4.02	$6.14 \times 10^6 \pm$90,000
	.0030	25.08±1.47	559.70± 6.69	534.62± 6.85	$5.94 \times 10^6 \pm$84,000
530	.0055	26.21±1.34	1445.39± 6.31	1419.18± 6.45	$7.86 \times 10^6 \pm$39,000

Table XIX

(a) P^{32} UPTAKE WITH TIME BY DYTISCUS SP. ADULTS IN THE AQUARIUM MICROCOSM

Sample Taken at (hours)	Sample Weight (gms dry)	Background Count c.p.m.	Gross Sample Count c.p.m.	Nett Sample Count (less background) c.p.m.	Counts/Minute/Gram at Zero Time
53	.0324	20.00±1.15	75.00± 2.50	55.00± 2.75	$.71 \times 10^5 \pm$ 3,540
248	.0310	20.14± .36	257.00± 2.68	236.86± 2.71	$3.48 \times 10^5 \pm$ 3,970
528	.0319	22.39±1.17	472.33± 3.58	449.93± 3.76	$6.53 \times 10^5 \pm$ 5,400
864	.1209	24.97±1.66	374.57±10.38	349.60±10.51	$2.01 \times 10^5 \pm$ 6,000
1200	.1363	28.39±1.54	363.28± 3.39	334.89± 3.73	$2.67 \times 10^5 \pm$ 2,600)
1200	.0868	31.18±1.89	130.85± 1.56	99.67± 2.45	$1.25 \times 10^5 \pm$ 3,000) Av. 1.96×10^5 2,800

(b) P^{32} UPTAKE WITH TIME BY NOTONECTA GLAUCA IN THE AQUARIUM MICROCOSM

9	.0550	25.74± .20	77.68± 1.22	51.94± 1.24	$.21 \times 10^5 \pm$ 500
53	.0170	22.34±1.12	251.75± 3.25	229.41± 3.61	$2.81 \times 10^5 \pm$ 4,430
168	.0589	25.26±1.26	780.94± 5.55	755.68± 5.69	$3.42 \times 10^5 \pm$ 2,580
528	.0600	27.22±1.50	409.72± 3.5	382.50± 3.81	$1.71 \times 10^5 \pm$ 1,710

Table XX

(a) P^{32} UPTAKE WITH TIME BY POLYCELIS NIGRA IN THE AQUARIUM MICROCOSM

Sample Taken at (hours)	Sample Weight (gms dry)	Background Count c.p.m.	Gross Sample Count c.p.m.	Nett Sample Count (less background) c.p.m.	Counts/Minute/Gram at Zero Time
9	.0034	16.31± .044	17.21± .54	.89± .54	$.03 \times 10^5 \pm$ 2,133
22	.0037	13.58± .43	17.95± .57	4.36± .71	$.26 \times 10^5 \pm$ 4,240
119	.0014	25.24± .84	35.85±1.13	10.61±1.41	$.62 \times 10^5 \pm$13,130
168	.0058	29.14± .92	40.84± .41	11.69±1.01	$.87 \times 10^5 \pm$11,970
363	.0038	27.14±1.38	40.03± .22	12.89±1.40	$1.81 \times 10^5 \pm$31,380
529	.0022	26.06± .89	35.46± .74	9.40±1.16	$1.98 \times 10^5 \pm$24,400
864	.0066	31.84± .88	47.69±1.51	15.86±1.74	$2.09 \times 10^5 \pm$23,000
1200	.0265	29.24±1.19	82.07±1.35	52.82±1.81	$2.08 \times 10^5 \pm$ 7,100

Table XXI

(b) P^{32} UPTAKE WITH TIME BY HYDRACARINA SPP. IN THE AQUARIUM MICROCOSM

Sample Taken at (hours)	Sample Weight (gms dry)	Background Count c.p.m.	Gross Sample Count c.p.m.	Nett Sample Count (less background) c.p.m.	Counts/Minute/Gram at Zero Time
1	.0031	17.06±.54	33.06± .67	15.99± .86	$6.93 \times 10^4 \pm 3,730$
7.5	.0016	14.58±.46	21.31±1.06	6.73±1.15	$5.84 \times 10^4 \pm 9,980$
26	.0017	13.81±.44	17.59± .35	3.78± .56	$3.47 \times 10^4 \pm 5,150$
53	.0010	-	-	-	-
168	.0019	-	-	-	-
363	.0027	-	-	-	-
528	.0013	-	-	-	-
864	.0039	-	-	-	-
1200	.0063	-	-	-	-

Table XXI

P^{32} UPTAKE WITH TIME BY GERRIS SPP. IN THE AQUARIUM MICROCOSM

Sample Taken at (hours)	Sample Weight (gms dry)	Background Count c.p.m.	Gross Sample Count c.p.m.	Nett Sample Count (less background) c.p.m.	Counts/Minute/Gram at Zero Time
8	.0046	14.94± .92	28.79± .23	13.86± .95	$.45 \times 10^5 \pm$ 3,080
22	.0033	13.43±1.21	37.02± .23	23.59±1.23	$1.08 \times 10^5 \pm$ 5,590
53	.0021	29.17± .94	36.94± .37	7.76±1.01	$.68 \times 10^5 \pm$ 8,820
119	.0068	41.85±1.32	92.72±4.19	50.87±2.75	$1.74 \times 10^5 \pm$ 9,350
363	.0035	28.09±1.05	59.50±1.02	31.42±1.46	$3.45 \times 10^5 \pm$17,000
530	.0063	25.22±1.12	101.87±3.19	76.65±3.38	$4.68 \times 10^5 \pm$ 2,060
864	.0021	26.15±1.15	45.65±1.53	19.51±1.91	$7.09 \times 10^5 \pm$70,000
1200					
Adult	.0074	26.22±1.47	65.35±2.68	39.13±3.05	$5.75 \times 10^5 \pm$ 4,500
Nymphs	.0042	28.98± .92	54.48± .13	25.51± .93	$6.33 \times 10^5 \pm$23,000

Table XXII

(a) P^{32} UPTAKE WITH TIME BY ENALLAGMA SPP. IN THE AQUARIUM MICROCOSM

Sample Taken at (hours)	Sample Weight (gms dry)	Background Count c.p.m.	Gross Sample Count c.p.m.	Nett Sample Count (less background) c.p.m.	Counts/Minute/Gram at Zero Time
8	.0254	28.50±2.69	125.73± 1.50	97.23± 3.08	1.19×10^5± 3,780
22	.0176	28.65±1.33	342.42± 5.65	313.77± 5.81	3.27×10^5± 6,050
53	.0229	22.80± .66	491.40±10.32	468.60±10.34	3.86×10^5± 8,220
103	.0226	15.42±1.24	400.27± 6.32	384.85± 6.45	6.06×10^5±10,160
119	.0117	15.85± .93	230.59± 7.27	214.73± 7.33	4.88×10^5±12,170
168	.0169	23.30±3.17	604.15± 2.48	580.85± 4.03	7.64×10^5± 5,290
363	.0154	24.19± .52	551.09±18.06	526.90±18.07	13.20×10^5±45,160
864	.0018	25.02± .89	39.33± .021	14.31± .91	8.83×10^5±56,250

(b) P^{32} UPTAKE WITH TIME BY EMERGING ENALLAGMA NYMPHS IN THE AQUARIUM MICROCOSM

22	.0088	20.00± .63	25.00± .79	5.00± 1.01	$.29 \times 10^5$± 5,930
119	.0055	16.00± .51	25.96± .82	9.96± .96	$.69 \times 10^5$± 6,680
144	.0068	29.10± .92	42.37±1.34	13.28± 1.34	2.38×10^5±24,140

Table XXIII

(a) P^{32} UPTAKE WITH TIME BY HYDROBIUS SP. IN THE AQUARIUM MICROCOSM

Sample Taken at (hours)	Sample Weight (gms dry)	Background Count c.p.m.	Gross Sample Count c.p.m.	Nett Sample Count (less background) c.p.m.	Counts/Minute/Gram at Zero Time
53	.0105	23.27±1.70	96.25± .49	70.48±1.25	$2.68 \times 10^5 \pm$ 4,760
216	.0102	20.71± .90	193.67±2.93	172.97±3.06	$9.95 \times 10^5 \pm$17,610
558	.0110	25.27±1.80	131.17±2.62	105.90±2.85	$7.70 \times 10^5 \pm$20,730

(b) P^{32} UPTAKE WITH TIME BY SIALIS LARVAE IN THE AQUARIUM MICROCOSM

96	.0155	20.81± .66	39.42± .88	18.61±1.10	$.51 \times 10^5 \pm$ 3,020
557	.0180	29.10± .92	190.45±4.26	161.35±4.36	$3.82 \times 10^5 \pm$10,320
1200	.0837	27.57± .87	771.78±7.72	744.21±7.77	$9.26 \times 10^5 \pm$ 9,670

(c) P^{32} UPTAKE WITH TIME BY ERPOBDELLA SP. IN THE AQUARIUM MICROCOSM

8	.0106	-	-	-	-
22	.0269	-	-	-	-
53	.0078	-	-	-	-
119	.0310	25.80± .84	64.40± .24	38.60± .87	$.61 \times 10^5 \pm$ 1,380
363	.0323	28.96±1.01	124.89± 3.95	95.93± 4.07	$1.32 \times 10^5 \pm$ 5,600
533	.1193	25.39± .87	794.70±11.36	769.31±11.40	$2.98 \times 10^5 \pm$ 4,420
864	.0833	25.96±1.75	536.12±13.99	510.16±14.10	$4.84 \times 10^5 \pm$11,430
1200	.2852	28.30±1.65	2018.5 ±24.88	1990.20±24.88	$7.27 \times 10^5 \pm$ 9,900

Table XXIV

(d) P^{32} UPTAKE WITH TIME BY DAPHNIA SPP. IN THE AQUARIUM MICROCOSM

Sample Taken at (hours)	Sample Weight (gms dry)	Background Count c.p.m.	Gross Sample Count c.p.m.	Nett Sample Count (less background) c.p.m.	Counts/Minute/Gram at Zero Time
1	.0004	15.09±1.39	40.78±1.23	25.69±1.85	$.87 \times 10^6 \pm 62,000$
22	.0007	16.33±1.08	129.04±1.83	112.71±2.12	$3.58 \times 10^6 \pm 64,000$
53	.0006	14.21±1.58	314.84±3.18	300.63±3.55	$7.71 \times 10^6 \pm 91,000$

Table XXIV

BIOLOGICAL VARIATION IN THE ^{32}P CONTENT PER GRAM OF HYPHYDRUS OVATUS INDIVIDUALS REMOVED AT 50 DAYS

Number	Weight	Background Count c.p.m.	Gross Count c.p.m.	Nett Count c.p.m.	c.p.m./gm at zero time
1	.0063	25.62± .81	54.19± .24	28.57± .84	$8.68 \times 10^5 \pm 28,200$
2	.0053	29.87± .94	49.31± .90	19.44±1.30	$6.67 \times 10^5 \pm 44,500$
3	.0053	27.56± .87	44.90±1.20	17.34±1.47	$6.31 \times 10^5 \pm 5,340$
4	.0061	29.54± .93	57.40±2.00	27.86±2.20	$7.94 \times 10^5 \pm 62,900$
5	.0067	29.33± .93	56.70±1.53	27.36±1.79	$7.10 \times 10^5 \pm 46,500$
6	.0053	28.53±1.01	58.65± .57	30.11±1.22	$9.88 \times 10^5 \pm 40,000$
7	.0054	31.89±1.00	49.10± .86	17.20±1.32	$5.79 \times 10^5 \pm 45,000$
8	.0058	35.70±1.13	58.11±1.75	22.41±2.08	$7.03 \times 10^5 \pm 65,000$
9	.0052	27.27± .86	49.76± .25	22.49± .90	$8.73 \times 10^5 \pm 35,000$
10	.0053	30.36± .96	50.66±1.20	20.30±1.52	$6.96 \times 10^5 \pm 52,000$
11	.0046	26.22± .48	29.40± .21	3.61± .52	$1.25 \times 10^5 \pm 21,000$
12	.0057	30.25± .96	57.30± .90	27.01±1.29	$8.62 \times 10^5 \pm 41,000$
13	.0047	29.85± .94	37.21± .83	7.36±1.26	$2.99 \times 10^5 \pm 51,000$
14	.0048	25.22± .80	46.94±1.17	21.73±1.42	$8.23 \times 10^5 \pm 53,000$
15	.0043	31.22± .99	41.58± .76	10.37±1.25	$4.19 \times 10^5 \pm 50,500$
16	.0044	29.61± .94	42.52± .95	12.91±1.33	$5.34 \times 10^5 \pm 55,140$
17	.0042	24.60± .78	41.93± .93	17.34±1.22	$7.51 \times 10^5 \pm 52,000$

Table XXV

P^{32} UPTAKE WITH TIME BY LIMNOPHILUS STIGMA IN A LABELLED AQUEOUS MEDIUM

Sample Taken at (hours)	Sample Weight (gms dry)	Background Count c.p.m.	Gross Sample Count c.p.m.	Nett Sample Count (less background) c.p.m.	Counts/Minute/Gram at Zero Time
12.5	.0593	30.16± .95	545.37±11.72	515.21±11.76	$1.85 \times 10^5 \pm 4,220$
56	.0633	33.71±1.07	615.67±26.39	581.96±26.41	$2.02\ 10^5 \pm 9,190$
120	.0811	23.73± .75	1054.48±16.67	1030.75±16.68	$2.32 \times 10^5 \pm 3,780$
168	.0912	33.81±1.07	446.43± 4.46	412.62± 4.59	$2.01 \times 10^5 \pm 2,230$
240	.1084	25.88± .82	956.24±17.46	930.36±17.48	$2.44\ 10^5 \pm 4,580$

P^{32} LOSS WITH TIME BY LIMNOPHILUS STIGMA IN AN UNCONTAMINATED MEDIUM

Sample Taken at (hours)	Sample Weight (gms dry)	Background Count c.p.m.	Gross Sample Count c.p.m.	Nett Sample Count (less background) c.p.m.	Counts/Minute/Gram at Zero Time
24	.0428	26.55± .84	208.42±7.19	181.87±7.62	$1.21 \times 10^5 \pm 5,060$
120	.0464	33.74±1.07	210.53±5.95	176.77±6.04	$1.08 \times 10^5 \pm 3,700$
216	.0299	29.37± .93	82.98± .83	53.61±1.25	$.82 \times 10^5 \pm 1,890$
360	.0858	36.03±1.14	431.34±7.21	395.32±7.30	$1.31 \times 10^5 \pm 2,430$

Table XXVI

P^{32} LOSS WITH TIME BY CYPRIS SPP. IN AN UNCONTAMINATED AQUEOUS MEDIUM

Sample Taken at (hours)	Sample Weight (gms dry)	Background Count c.p.m.	Gross Sample Count c.p.m.	Nett Sample Count (less background) c.p.m.	Counts/Minute/Gram at Zero Time
0	.0010	33.52±2.18	455.17±7.84	421.65±8.14	$15.06 \times 10^6 \pm 290,000$
44.5	.0015	34.56±1.48	554.19±5.90	519.63±6.09	$12.37 \times 10^6 \pm 145,000$
144	.0022	32.05±2.04	400.79±4.37	368.74±4.82	$5.99 \times 10^6 \pm 78,000$
288	.0039	28.63±1.40	508.29±6.07	479.66±6.23	$4.92 \times 10^6 \pm 64,000$
480	.0025	26.03±1.01	134.63±1.51	108.59±1.81	$2.86 \times 10^6 \pm 48,000$